The Journal of Parasitology

Volume 29 APRIL, 1943 Number 2

THE DEVELOPMENT OF THE LARVAL STAGES OF *PLAGIORCHIS MURIS* TANABE, 1922, IN THE FIRST INTERMEDIATE HOST[1]

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During the summer of 1941 we undertook a study at the University of Michigan Biological Station of the development of the larval stages of *Plagiorchis muris* in natural infections in the common beach snail, *Stagnicola emarginata angulata* (Sowerby). Very little is known of the mother sporocyst or of the development of the daughter sporocysts of the Plagiorchioidea. *P. muris* was chosen for our first study on this group because its larval stages occur in very high incidence in both juveniles and adults of *S. emarginata angulata* in convenient locations near the Biological Station.

Information on the life cycle of *P. muris* in the United States is available in the literature from the work of McMullen (1937a). He studied the mature daughter sporocysts and cercariae and noted that precocious development of the metacercariae frequently occurred inside the sporocysts (McMullen, 1938). Adults were obtained by feeding to pigeons, rats, mice and man (McMullen, 1937b) either precociously developed metacercariae from naturally infected snails or those from experimentally infected insect naiads. Natural infections have been reported in the robin, herring gull, night-hawk, spotted sandpiper, and the muskrat. No experimental infections of the first intermediate hosts have been carried out with this species and nothing has been reported on the mother sporocyst stage, the development of the daughter sporocysts, or the germ cell cycle.

To obtain the early developmental stages of the sporocysts of *P. muris,* we examined between July 21 and September 4, 1941, several hundred juveniles and some adults of the common beach snail, *S. e. angulata* from a beach on Burt Lake (the Burt Lake area, Cort et al, 1937) where the cercariae of this species had been found for several years in very high incidence. The other larval trematodes in this host in this area were chiefly strigeids, schistosomes, notocotylids, and echinostomes. The early developmental stages of members of these groups can be distinguished without the slightest difficulty from those of the plagiorchiids. The only other larval stages of plagiorchiids that have been found in a large series of examinations of this host in this area have belonged to *P. proximus* Barker, 1915, *P. micro-*

Received for publication, February 19, 1942.

[1] A contribution from the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University. This is the third of a series of studies on the development of the sporocysts and the germ cell cycle of digenetic trematodes. The earlier investigations dealt with the strigeids (Cort and Olivier, 1941) and a schistosome (Cort and Olivier, 1943).

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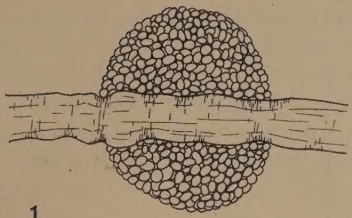
canthos Macy, 1931, and *Cercaria talboti* (McMullen, 1938). The last two were so very rare that they can be disregarded. The mother sporocysts and the early stages of the daughters of *P. muris* can be distinguished from those of *P. proximus*, which will be covered in a later paper. We can, therefore, be practically certain that the stages described in this paper belong to *P. muris*.

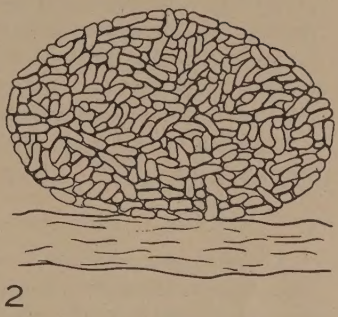
The snails to be examined were carefully pulled out of their shells, and teased apart with very sharp needles under a binocular dissecting microscope. Any structures suspected of being mother sporocysts or immature daughter sporocysts of plagiorchiids were carefully freed from the tissues and removed to a slide for study. The preparations were made either with tap water or 0.5 per cent saline, and were studied with the compound microscope under magnifications up to almost 1000. By examining large numbers of snails we were able to secure and study a considerable series of the developmental stages of the sporocysts of *P. muris*.

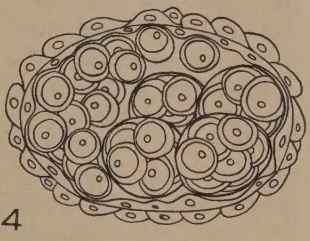
MOTHER SPOROCYST STAGE

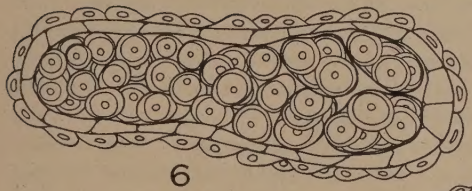
In the examinations of the snails we looked carefully through all the tissues and especially along the whole length of the intestine to see if we could find mother sporocysts of *P. muris*. Since we had no idea of what the mother sporocyst would be like in this species, we could only study any suspicious looking structures that were found. On July 25, we found in two juvenile snails peculiar masses attached to the outer wall of the intestine. They were composed almost entirely of large numbers of what appeared to be daughter sporocyst embryos. During the course of our examinations about 20 such masses were found and further studies convinced us that they represented late stages of mother sporocysts of *P. muris*. When these mother sporocysts were found in the snails no other stages were present except in a very few cases which were obviously double infections.

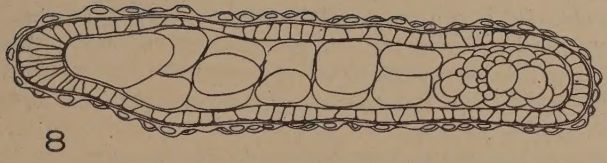
These mother sporocysts were round or oval with the greatest diameter varying from 0.5 to 1.7 mm (Figs. 1 and 2); their thickness was distinctly less than their width, and they seemed to be roughly lens-shaped. They were solid, discrete structures with a regular outline and adhered firmly to the outside of the intestine, being attached usually by an edge but sometimes by one of their broad surfaces. They were found both on the first part of the intestine before it reaches the digestive gland and on the last part which passes from the digestive gland to the anus. When present on the middle portion of the intestine, which is imbedded in the surface of the digestive gland, they were imbedded in the glandular tissue and were often broken in pulling the intestine free. Some of these mother sporocysts were freed unbroken, since the daughter sporocyst embryos, of which they are composed, were tightly held together. In surface view, the masses appeared to be made up of numerous compartments (Figs. 1 and 2), which on closer inspection turned out to be closely packed daughter sporocyst embryos. It was estimated that each of the mother sporocysts was composed of from 300 to 500 of these embryos. All those in the same mother were at about the same stage of development; in the smallest they were round or oval, and in the largest, they were much larger, elongate sacs, which showed considerable mobility when freed from the mass. No orderly arrangement of the embryos could be seen in the mother sporocyst masses. The daughter sporocyst embryos of all sizes (Figs. 3–8) were covered by an outer coat of irregular cells; and, in fact, the whole matrix of the mother sporocyst was composed of this one











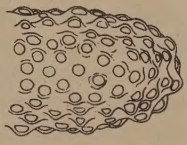


FIG. 1. Mother sporocyst mass attached to intestine of snail. Greatest dimension 0.5 mm. One of youngest stages found with daughter sporocyst embryos very immature (at about the stages shown in Figs. 3 and 4).

FIG. 2. Mother sporocyst mass attached to intestine of snail. Length 1.7 mm. Daughter sporocyst embryos at about the stage of Fig. 8.

FIGS. 3 and 4. Very young embryos separated from the mass of the youngest mother sporocyst of our series. Length, Fig. 3, 0.3 mm; Fig. 4 to scale.

FIGS. 6 and 7. Stages in development of daughter sporocyst embryos from mother sporocyst masses. Length, Fig. 6, 0.1 mm; Fig. 7, 0.13 mm.

FIG. 8. Daughter sporocyst embryo about ready to escape from mother. Note the fully developed outer coat, the paletot, and the discrete germ mass. Length, 0.2 mm.

FIG. 9. Surface view showing the configuration of the paletot.

type of cell. This outer coat was also present in all the later stages of the daughter sporocysts of this species, including those that were mature or old. It appears to be the same as the outer layer described by some of the earliest workers on larval trematodes on the sporocysts of certain species of xiphidiocercariae. It has also been reported more recently by several workers in descriptions of daughter sporocysts of this group. This outer layer was very aptly called the paletot (overcoat) by Bieringer (1885).

STAGES OF DEVELOPMENT OF THE DAUGHTER SPOROCYST EMBRYOS

The youngest daughter sporocyst embryos removed from mother sporocysts, which were at about the stage of Fig. 1, were round or slightly oval and measured from about 0.030 to 0.040 mm in diameter (Fig. 3). The true sporocyst wall at this stage consisted of a thin membrane composed of flattened cells. The contents consisted of a mass of separate germ cells, which were so closely crowded together that they looked almost like the morula stage of an embryo. In the same mother sporocysts with these smallest embryos there were also slightly larger, distinctly oval daughter sporocyst embryos about 0.050 to 0.070 mm in diameter (Fig. 4). In these the wall was slightly thicker and its cellular elements more distinct. The germinal material was not as tightly packed and two to four cercarial embryos at a very early stage of development were present at one end. In two somewhat older mother sporocysts the daughter sporocyst embryos varied from about 0.050 to 0.115 mm in length and from about 0.034 to 0.057 mm in width. The largest of them were distinctly more elongate (Fig. 6). The true wall of the sporocyst was much thicker and was composed of a single layer of large cells. The number of cercarial embryos that had started to develop had increased to about 5 to 7. They were located at one end of the body cavity and the rest of the cavity was filled with single germ cells.

At a somewhat later stage (Fig. 7) the number of developing embryos varied from 6 to 10, and the largest of them were much further along in development so that their individual cells could not be so clearly distinguished. In a mother sporocyst, containing many embryos at about this stage, they measured 0.064 to 0.160 mm in length and 0.040 to 0.061 mm in width. In another somewhat older mother sporocyst all the daughters were larger and distinctly elongate, varying in length from 0.140 to 0.160 mm. In these daughters the sporocyst wall had increased in thickness especially at the ends, the cells having become very distinct. Each daughter sporocyst contained 6 to 8 rather well developed cercarial embryos, and some smaller embryos.

In the oldest mother sporocysts observed the daughters (Fig. 8) were elongate, very mobile, when freed, and appeared capable of migration. In one such mother they varied from 0.120 to 0.200 mm in length and from 0.035 to 0.048 mm in width. In others, some of them were considerably larger. They were very mobile and could extend and contract their bodies, causing the contents of the well defined body cavity to move freely back and forth. The paletot was entirely separated from the few matrix cells that still remained in the small spaces between the crowded daughter sporocysts for the mother sporocyst had become almost entirely a mass of daughter sporocysts. The cells of the paletot were crowded close together and appeared on surface view of the sporocysts as elevations or bosses (Fig. 9). The true body wall of the daughter sporocyst had become much thicker and its layer of cells more

prominent, especially at the ends where distinct thickenings occurred. In one end of the body cavity usually the more mobile anterior end, there was present a discrete, definitely organized germ mass (Fig. 8) made up of multicellular units of different sizes, and single cells. Besides a few very small embryos near the germ mass, the body cavity contained several larger cercarial embryos.

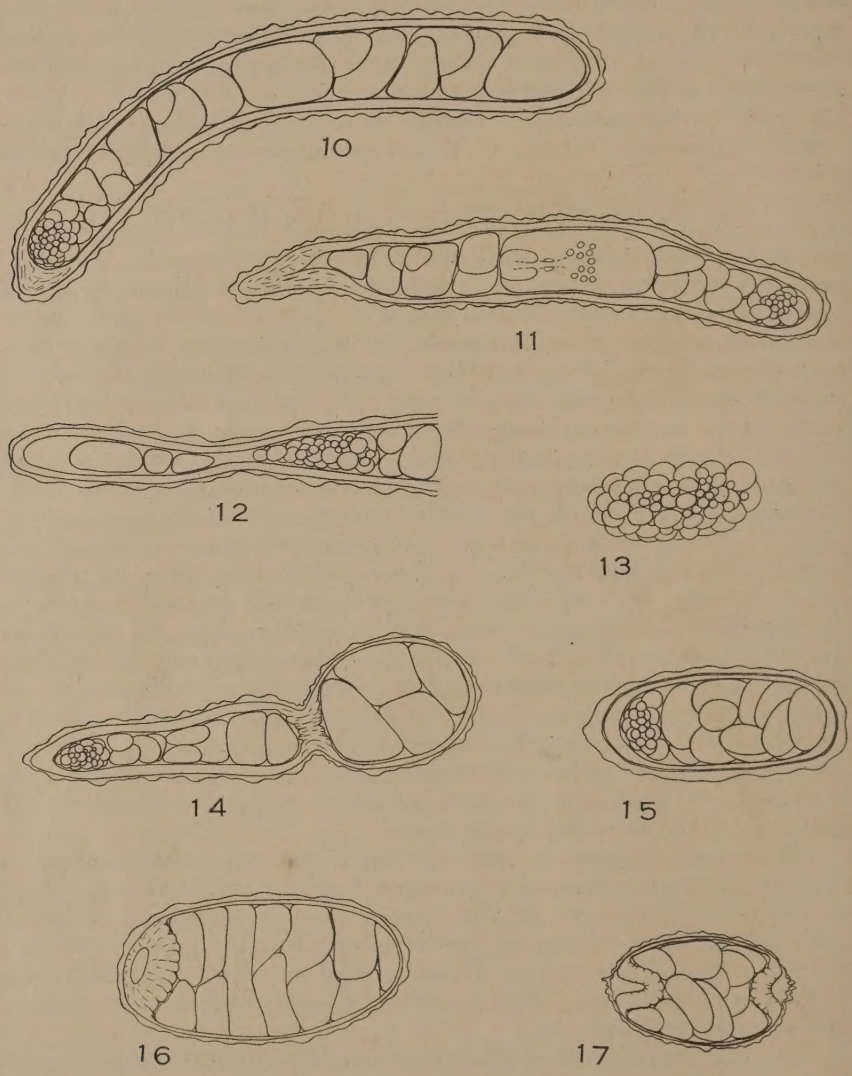
In one immature daughter sporocyst it was possible to make out the excretory system, which consisted of two pairs of flame cells attached to two entirely separate tubules.

MIGRATING DAUGHTER SPOROCYSTS (FIGS. 10 AND 11)

The first clue to the further development of the daughter sporocysts of *P. muris* came from the finding of sporocysts, exactly like the oldest in the mother sporocyst masses, scattered throughout the snail tissues. Some were found on the wall of the stomach and intestine, along the blood vessels leading from the heart, and along the ducts and vessels that pass out of the digestive gland; but most of them were located in the digestive gland along the ducts and vessels that extend to every part of this organ. In two cases, large numbers of these migrating daughter sporocysts were found in the digestive gland and on the viscera while the remnant of a mother sporocyst was still adhering to the intestine. In other infections no trace of a mother sporocyst remained and numerous daughters were apparently migrating through the snail. In still others, only a part of the sporocysts observed were in the migrating stage and the rest were inactive. It appears, therefore, that when the daughter sporocysts break away from mothers located on the parts of the intestine in front of the digestive gland, they migrate extensively along the blood vessels, the reproductive ducts, that come from the hermaphroditic organ inside the digestive gland, and along the ducts from the digestive gland itself. These daughter sporocysts seem to be very effective migrators on account of their great power of extension and contraction and the roughness of their outer surfaces produced by the projecting cells of the paletot. They seem, also, to carry out the complete migration in a comparatively short time, since as already noted, they may reach all parts of the digestive gland before all have escaped from the mother sporocyst mass.

The migrating daughter sporocysts of *P. muris* were very mobile, being able to extend themselves to at least twice their normal length (Fig. 12). As stated above, it is estimated that between 300 and 500 of them are produced by each mother sporocyst, and in one infection an incomplete count gave 265. They varied considerably in size; in one infection they had a range from 0.192 mm by 0.077 mm to 0.512 mm by 0.070 mm. Migrating daughters measured from other infections also fell within this range.

In structure, the migrating daughter sporocysts (Figs. 10 and 11) have advanced but little beyond the largest stages obtained from the oldest mother sporocysts. The body cavity contained 12 to 15 fairly well developed cercarial embryos, some that were still very small, and the germ mass. The largest embryo, in which sometimes the suckers and other structures of the cercaria could be made out (Fig. 11), was usually located at about the middle of the sporocyst and was sometimes as much as 0.1 mm in length. The germ mass was usually at the more attenuated anterior end of the sporocyst, sometimes next to the end, but frequently separated from it by one or a few embryos. In a few cases, it was located at the posterior end or near the



FIGS. 10 and 11. Daughter sporocysts in the migrating stage. Length, Fig. 10, 0.5 mm; Fig. 11, 0.44 mm.

FIG. 12. Attenuated anterior end of daughter sporocyst in migrating stage.

FIG. 13. Germ mass from migrating daughter sporocyst. Length, 0.075 mm.

FIG. 14. Daughter sporocyst in transition from migrating to fixed stage.

FIGS. 15, 16 and 17. Daughter sporocysts just after attachment to snail's tissues. Note shortening and thickening and growth areas at ends. All about 0.2 mm in length.

middle of the body cavity. The germ mass had increased in size and was sometimes as much as 0.075 mm in length. It appeared as a definite, discrete structure like the germ masses of the strigeids (Cort and Olivier, 1941), moved as a unit, and was surrounded by its own membrane (Figs. 8, 10, 11, 12, and 13). It was composed of both single and many-celled components, the largest of which had the same size and structure as the smallest free cercarial embryos. It is suggested that, as in the strigeids, the breaking off of the largest components of the germ mass continually adds to the number of free embryos inside the sporocyst.

When the mother sporocysts were located on the part of the intestine imbedded in the digestive gland, migration of the daughters appeared to be distinctly limited. In some of these cases it appeared that part of the daughter sporocysts had migrated away from the mother into all parts of the gland and that others had developed in a mass at the position of the mother sporocyst. In other cases only a few of the sporocysts appeared to have migrated and in a few cases all had developed in a single mass without leaving the mother. In fact, in one case mature daughter sporocysts were found in a large mass between the foot and gland attached to the intestine. We interpreted this also as a case of development without migration from the mother sporocyst.

STAGES OF SPOROCYST DEVELOPMENT AFTER MIGRATION

In some immature infections in which migrating daughter sporocysts were present, other sporocysts of an entirely different type were found. They were non-motile and were firmly attached along the ducts and vessels in the digestive gland, in front of this organ in the region near the heart, and on the walls of the stomach and intestine. They varied greatly in shape and some were very irregular. They appeared to be firmly attached along their long axis to the hosts' tissue, but just how this attachment was effected was not clear. In some infections all the sporocysts appeared to have just stopped migrating and become attached.

Some of these immature attached daughter sporocysts were oval and considerably shorter and thicker than the migrating stage (Figs. 15, 16, and 17). Fig. 14 appears to show a transition stage from the shape of the migrating sporocysts. In many of them the true sporocyst wall at either or both ends showed thickened areas with the cellular layer greatly enlarged (Figs. 16 and 17). At the centers of these thickened areas there were usually invaginations which gave them a sucker-like appearance. These sporocysts were crowded with cercarial embryos which were so tightly packed that no space whatever was left between them and their shapes were modified by contact with each other (Fig. 16). The largest of the cercarial embryos showed the suckers and other structures of the body, but were still very immature.

Also, in these immature infections some sporocysts were present, which bore projections that appeared to arise from the centers of the thickened areas at the ends. Their projections were sometimes small and bulb-like (Fig. 18), but frequently larger and either round or elongate (Figs. 19 and 20). Some of them were made up entirely of sporocyst wall (Figs. 18, 19 and 20), but in many cases the body cavity containing cercarial embryos extended into them (Figs. 22 and 23). In some of these immature sporocysts the projections were round, crowded with embryos and almost the same size as the original sporocyst (Figs. 21 and 25), giving the appearance of fission or budding. Frequently, the two parts filled with embryos were separated by a tube of varying length containing no embryos (Fig.

24). In some cases even, sporocysts were divided into three approximately equal divisions (Fig. 26). In fact, in immature infections of this type, in which none of the cercarial embryos were even approaching maturity, sporocysts of a wide variety of shapes, some of which were very bizarre, were found (Fig. 31). In some of the larger of the irregularly shaped sporocysts the cercarial embryos showed the beginnings of a tail. Sometimes, in an infection there would be found some of the irregularly shaped sporocysts of the type just described and others that were larger and more regular in shape with some cercariae approaching maturity.

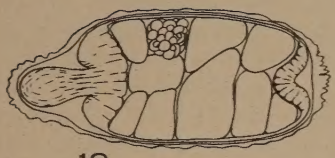
Except where it was torn off by manipulation, each sporocyst was surrounded by the outer layer originally formed of mother sporocyst cells, the paletot, which in places was not closely attached to the true sporocyst wall (Figs. 18, 21 and 25). In a few cases these developing stages were found in sporocysts that had never migrated from the mother, especially when the mother sporocysts were imbedded in the digestive gland.

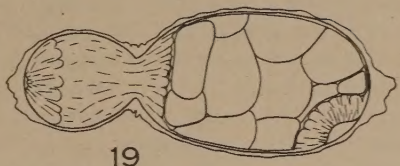
Only a few infections were found in which the daughter sporocysts had developed beyond the small irregular stage just described, and yet had not begun to produce mature cercariae. In one such infection in an adult snail the oldest cercariae had well developed tails, but were still immature. The sporocysts in this case varied in size from 0.26 by 0.06 mm to 0.68 by 0.12 mm, with most of them under 0.40 mm in length. They showed a variety of shapes (Fig. 32) but were much more regular than those shown in Fig. 31, and were filled with embryos. It can be seen by comparing the outline drawings of sporocysts from this infection (Fig. 32) with those shown in Fig. 31, that they were no longer than those of the earlier stage, but differed chiefly in having a greater width with no projections or narrower parts that were not filled with embryos.

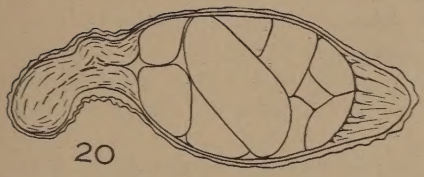
In the infection just discussed we examined almost every sporocyst, but could not find a single case in which two or more sporocysts were enclosed in a single paletot. In fact, after looking exhaustively in a large series of infections, both those containing only immature cercariae and those with mature cercariae, we were able to find only a very few cases which suggested that two or more separate sporocysts were enclosed in a single paletot; and only in the three cases shown in the drawings (Figs. 27, 28, and 29) could we be reasonably certain that the sporocysts inside the same paletot were definitely separated.

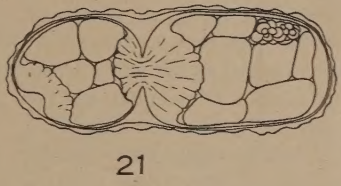
MATURE SPOROCYSTS

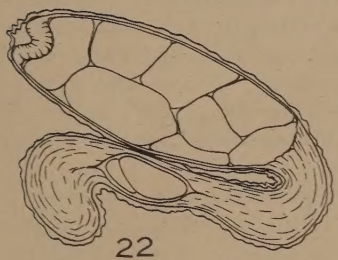
Large numbers of young mature infections of *P. muris* were found, especially in the juvenile snails. They were characterized by the presence of small numbers of mature cercariae. In most cases the sporocysts were somewhat larger in size than in infections such as that just described in which none of the cercariae were yet mature. However, in a few cases where mature cercariae were present the size was somewhat less, as for example, one in which the sporocyst size varied from 0.33 by 0.12 mm to 0.61 by 0.19 mm. The sporocysts at this stage were plump and filled with embryos, and the few mature cercariae that were present moved actively. Careful examination disclosed the presence in each sporocyst of a single germ mass as well as numbers of small cercarial embryos in different stages of development. The paletot had become a thicker, firmer membrane and occasionally showed some orange pigmentation and the true sporocyst wall was relatively thinner than in the earlier

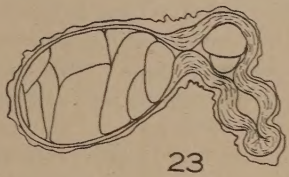


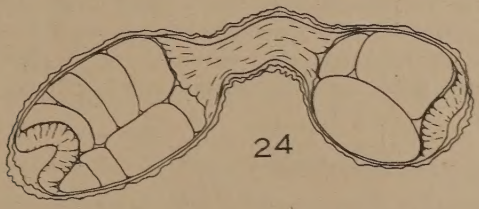


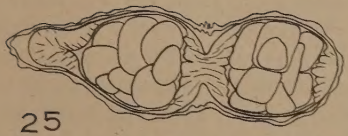


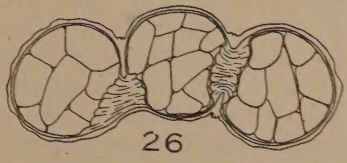












FIGS. 18–26. Very immature daughter sporocysts in growth stages. Note areas of growth and variety of shapes assumed. Length of Fig. 18 is 0.26 mm. Figs. 18 to 24 drawn to scale.

stages. The majority of the sporocysts at this stage were quite regular in shape, but a number were distinctly irregular (Fig. 33). The end from which the cercariae escaped was more pointed than the other, but the birth pore could not be made out. Young mature sporocysts varied greatly in size, both in the same infection and in different infections. A few were less than 0.5 mm in length and occasionally the largest was over 1 mm. Most, however, were between 0.5 and 0.8 mm in length and from 0.1 to 0.2 mm in their greatest diameter. All but the three largest of the outline drawings of Fig. 33 were made from young mature sporocysts.

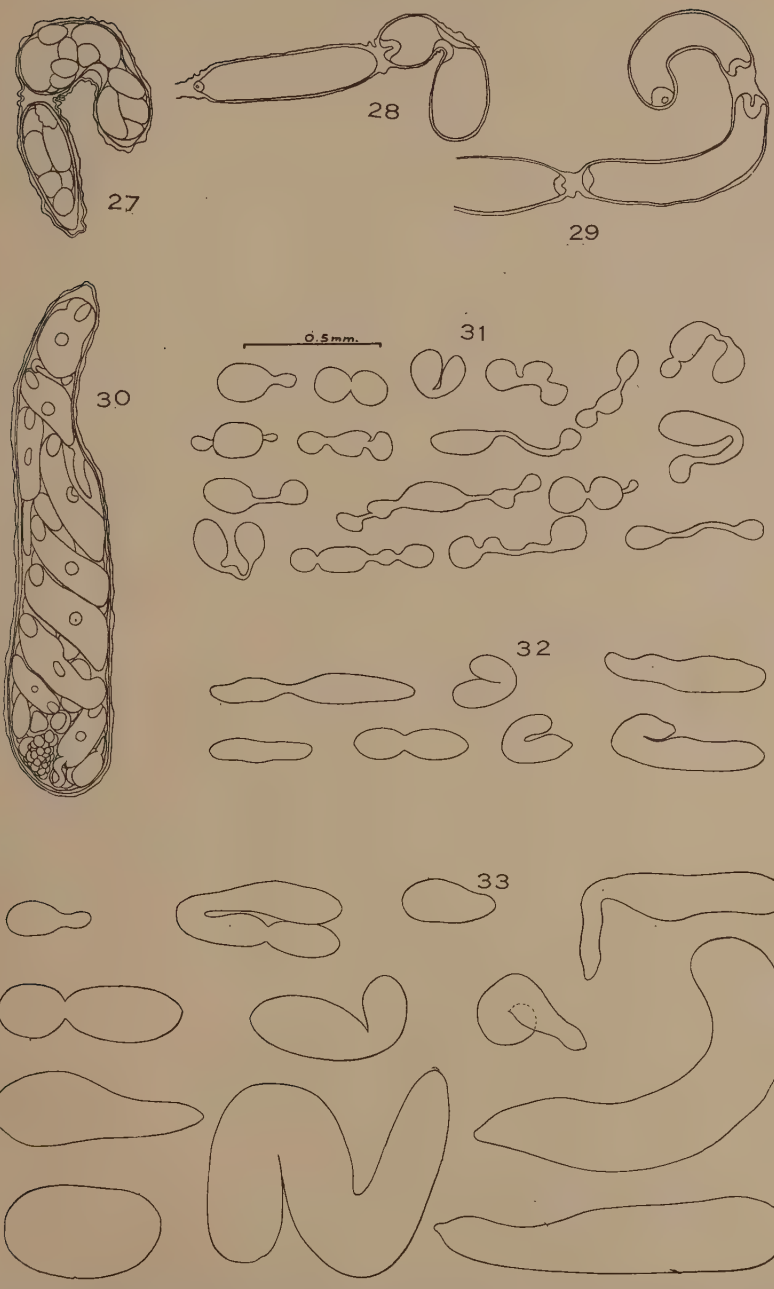
Older daughter sporocysts with larger numbers of mature or almost mature cercariae were larger (Fig. 30). The paletot was still thicker and contained a considerable amount of orange pigment. The number of mature, or almost mature, cercariae in each sporocyst was larger, usually being more than 10. The birth pore was located at the pointed end but could only be seen clearly when cercariae were escaping. Developing embryos at all stages were present and the germ mass appeared unchanged (Fig. 30).

In the oldest infections which were found in old snails in September some of the sporocysts were from 1 mm to 1.6 mm in length. Occasionally, still larger ones were found, the largest measured being almost 3 mm in length and containing about 40 mature or almost mature cercariae. However, in most mature infections including the oldest, the sporocysts averaged under 1 mm in length and in some cases, were less than 0.5 mm. Thus, a considerable variation in size of sporocysts between infections was found, and the variation in a single infection was also large. Certainly, if the sporocyst size for other species of plagiorchiids varies as much as that for *P. muris,* measurements such as those given in most descriptions have little validity as specific characters.

Even in the very oldest infections in the old snails examined in September, there was no evidence of the exhaustion of any of the daughter sporocysts. They were especially heavily pigmented and, in some cases, not nearly as crowded with cercariae and embryos as the younger ones. Nevertheless, there were always large numbers of mature cercariae still present and germ masses and very small embryos could still be seen. In fact, in several old infections in which precocious development had occurred, germ masses and small embryos could be found crowded in among the metacercariae.

RELATIONS OF INFECTIONS OF *P. muris* TO THE SNAIL INTERMEDIATE HOST

From the studies of the summer of 1941 we obtained some information that supplemented our earlier data on the relations of infections of the larval stages of *P. muris* to the snail host, *S. emarginata angulata* (cf. Cort, 1941). Infection of the snails must begin to take place in the very youngest juveniles since immature daughter sporocysts were found in very small juveniles and a few young mature infections were present by the first of August. In fact, in our examinations of 404 juveniles collected from the Burt Lake area on July 25, 1941, and examined from that date until about the middle of August, we found 23 (5.7 per cent) infections of *P. muris* with some cercariae fully or almost fully developed. Also, in a collection of juveniles made from the same area on August 2, 1938, five infections were mature or almost mature and from three of these cercariae were escaping. Since juveniles of this snail species apparently do not usually hatch on the beaches in any number



FIGS. 27–29. Drawings showing the three cases in which more than one daughter sporocyst was found inside the paletot.

FIG. 30. Mature daughter sporocyst. Length 1 mm.

FIGS. 31, 32 and 33. Outline drawings at about the same scale of the stages of development of the daughter sporocysts; Fig. 31, earliest growth stage; Fig. 32, stages in which cercariae are not yet mature; Fig. 33, young mature and mature stages.

before July 1 (Cort et al, 1940), it seems evident that the development up to the time that the first cercariae are mature may require only a little over a month. New infections continue to enter the juveniles at least until after the first of August since as many mother sporocyst stages were found in the collection of August 21 as in that of July 25. In the various collections of juveniles that have been made in late August and early September of several different summers from the Burt Lake area a high percentage of infection with *P. muris* was always found. This percentage was slightly greater in the collections of adult snails which developed from this same generation of juveniles which were made in June of the next year, increased in later summer collections of adults, and was at its peak in the collections of late August and early September (Cort, 1941). Also, in the adult snails examined from this area in the summer of 1941 mother sporocysts and early developmental stages of daughter sporocysts of this species were found. In these collections, even those made late in the summer, we could distinguish the older infections that had been carried over the winter from those that had entered the same year, since the sporocysts in them were larger, more heavily pigmented, and contained larger numbers of mature cercariae. As noted above, even the oldest infections found in old snails in early September were still actively producing cercariae and the sporocysts contained the youngest embryos and germ masses. It appears, therefore, that infections of *P. muris* enter the snails of this species throughout both summers of their life. Therefore, the oldest infections found in old snails in early September, which were still actively producing cercariae, must have been more than a year old. It can be suggested, therefore, that these snail hosts acquire infections of this trematode from their youngest juvenile stages until they are well past maturity, and that even those acquired by the youngest juveniles probably continue to produce cercariae until the natural death of their host.

Another striking point was the very small amount of injury produced in the snails by the sporocysts of *P. muris* even in old, heavy infections. We have no evidence of their effect on the reproductive organs; but the digestive gland showed but slight injury compared with that produced by old infections with strigeids, schistosomes, and echinostomes. This appears to be due to the fact that the sporocysts are chiefly attached to the ducts and vessels of the gland and are immobile except in the migrating stage. Also, there is no evidence that infected snails are killed by infections of *P. muris* even over the winter, since as noted above, there is a steady increase in the incidence of this parasite all through the life of the snails. On the other hand, striking reductions of incidence of infection of certain echinostomes and strigeids in older as compared with younger snails have been interpreted as showing a differential death rate of the infected snails (Cort et al, 1939; Cort, 1941); also, in infections of these forms considerable damage to the digestive gland of the host is apparent.

DISCUSSION

The most interesting points brought out in the studies of the development of the larval stages of *P. muris* in naturally infected snails have been the following: (1) the finding of mother sporocysts which are entirely different from any previously described for other trematodes; (2) the discovery that the outer membrane, the paletot, of the daughter sporocysts is derived from the wall of the mother sporocyst; (3) the discovery of the method of migration of the young daughter sporocysts and

the description of the migrating stage; (4) the finding of immature sporocysts of irregular shapes with projections formed from areas of growth at their ends; (5) the demonstration of a discrete germ mass in the young daughter sporocyst which persists throughout its whole life; and (6) the tracing of the seasonal cycle of infection in relation to the life cycle of the snail host.

Although in the superfamily PLAGIORCHIOIDEA, as defined by McMullen (1937c) to include all forms with true xiphidiocercariae, about fifty life cycles have been worked out to the extent of connecting cercariae with adults, surprisingly little is known of the early stages of development in the snail intermediate host. In only a few of the known life cycles has the intermediate host been infected experimentally and we have found descriptions of mother sporocysts for only four species, all after experimental infection. Three of these belong to one group, the RENIFERINAE (Talbot, 1933; Byrd, 1935; Walker, 1939) and the other is *Plagitura parva* (Stunkard, 1936). In all these cases the mother sporocysts are described as thin-walled sacs in which daughter sporocysts develop and from which they later escape and migrate to the position where they continue their development. The outer coat or paletot is not shown for the daughter sporocysts of these species.

The mother sporocyst stages of *P. muris* described above are entirely different from any of those previously described for the PLAGIORCHIOIDEA. They appear to represent only relatively late phases of mother sporocyst development. Therefore, we can only make certain very tentative suggestions in regard to their development. In the first place, it seems practically certain from the position of these mother sporocysts on the outside of the intestine that infection of the intermediate host is by ingestion of embryonated eggs. Another point worth stressing is that the germinal material of the early mother sporocyst of this species must have been so organized that all the embryos started to develop at about the same time. Only in this way can the fact be explained that all the daughter sporocyst embryos in any mother sporocyst are in about the same stage of development, and escape from the mother at about the same time. In most other mother sporocysts that have been described the evidence is clear that daughter sporocysts are produced in series over a period of a number of days or even weeks.

It also seems difficult to fit in the behavior and position of the irregular matrix cells of the mother sporocysts of *P. muris* with anything previously known about the development of this stage in other trematodes. These cells form an outer layer around the daughter sporocyst embryos that persists throughout their further development. Such an outer layer (paletot) in daughter sporocysts of certain xiphidiocercariae seems to have been first described by Steenstrup (1842) for the sporocysts of *Cercaria armata*. Moulinie (1856), de Filippi (1857) and Wagener (1857) also saw it on immature sporocysts in infections not far enough along to be identified. Biehringer (1885) and Schwartze (1886) also reported the presence of this layer on the sporocysts of *C. armata* and clearly showed its division into separate cells. The origin of this layer was considered by several authors. De Filippi concluded that it was the remains of a mother sporocyst that had produced a single daughter within itself. Leuckart (1863) suggested that it did not belong to the sporocysts at all but was formed from the host's tissue. Biehringer (1885) accepted this view and presented evidence which he thought demonstrated that the paletot was formed from the cells of the snail's blood which attached themselves to the outside of the

sporocyst and spread themselves out into an epithelial layer. The views of this author on the origin of the paletot were accepted by Leuckart (1889, p. 107) and Braun (Pagenstecher and Braun, 1879–1893, p. 811).

More recent references to the paletot are surprisingly few. Dubois (1929, p. 6) showed what is probably this layer in a cross-section of the wall of the sporocyst of a xiphidiocercaria (*Cercaria helvetica* V), and Harper (1929) described it for the sporocysts of his Cercaria X. Brooks (1930) also described an outer "sheath composed of epithelial cells" around the sporocysts of three xiphidiocercaria species. The only other recent reference we have been able to find to the paletot is in the monograph of Wesenberg-Lund (1934). This author mentioned the presence of a "yellow paletot" on the sporocysts of *Haplometra cylindracea* which he found in *Lymnaea stagnalis*. He also described it for the sporocysts of *Cercaria cellulosa* as a very thick layer consisting of flattened cells (Pl. XIV, Fig. 8). In this case cercariae were found between this layer and the outer wall of the sporocyst. The small number of recent references to the paletot is perhaps due to the failure of most authors who describe xiphidiocercariae to pay any attention whatever to the structure of the sporocysts.

We have been unable to find in the literature any descriptions of immature sporocysts of xiphidiocercariae that resemble the migrating stage of *P. muris*. As mentioned above we concluded from our observations that when the mother sporocyst masses are located on the parts of the intestine in front of the digestive gland, the daughter sporocysts migrate widely and become attached chiefly along the ducts and vessels, both between the foot and the gland and within the digestive gland itself. In fact, when the ducts and vessels in the digestive gland were carefully pulled free from the parenchyma practically all the sporocysts present in the gland would remain adhering to them. On the other hand, when the mother sporocysts were located on the surface of the digestive gland migration was much more limited.

When we first found infections containing the small irregular sporocysts of the type shown in Figs. 18 to 26 and 31, we had great difficulty in fitting them into the scheme of development. It was evident from the fact that the cercarial embryos in them were still very immature, that they represented stages just beyond the migrating daughter sporocysts. At first we were inclined to the opinion that they represented a phase of division by budding and fission. It seemed evident that if the irregular, immature sporocysts of *P. muris* were really in a stage of division, the next stages would show numerous cases of completely separated sporocysts within a single paletot. Exhaustive search in which large numbers of sporocysts of different ages were observed in a variety of different infections gave us only the three cases that have been figured (Figs. 27, 28, and 29) in which more than one sporocyst was enclosed by a single paletot. Also, in these examinations nothing else was found that suggested that there had been fission or budding. Furthermore, each sporocyst contained only one germ mass exactly like that found in the migrating stage. It would be very difficult to postulate a method for providing new germ masses for sporocysts produced by budding or fission. We were forced, therefore, to the view that in the usual course of the development of the larval stages of *P. muris* only one mature daughter sporocyst is formed from each embryo of the mother sporocyst.

Later, we became convinced that these irregularly shaped immature sporocysts are really undergoing changes by which the size is increased to make room for the

rapidly increasing embryonic material. According to this view, soon after the migrating stages reach suitable locations they cease movement and become attached to the hosts' tissues. Then the ends of the sporocysts become regions of growth and form projections. The lumina of the projections remain connected with the main body cavity of the sporocysts and embryos are gradually pushed into them. As the projections grow larger they are occupied by more and more cercarial embryos and eventually fill out to the same diameter as the original sporocyst. In addition, new projections may in turn grow out from growth areas at their ends. As larger numbers of new embryos are produced from the germ masses and as those already present increase in size the irregular shaped sporocysts are filled out and become more regular in shape, although still showing some indications of the shapes they had assumed during the growth period (Fig. 32). In fact, some trace of these unusual shapes may still appear in mature sporocysts (Fig. 33). This early growth may, therefore, be considered as a combination of an increase in size of the sporocysts by the growth of projections from the growth areas at the ends and a rapid increase in the amount of germinal material, which crowds into all available space.

It appears that the time from the migration of the daughter sporocysts until the first cercariae become mature is very short, perhaps only a few days. Evidence for this is that the oldest cercarial embryos in the migrating sporocysts are surprisingly large and well developed. Also, in the stages just after migration they soon show the development of the tail and body structures. In several infections, a few mature cercariae were present in some of the sporocysts while others were in the stages showing projections from the growth areas of the wall. In addition, only a very few infections were found in all our examinations in which all the cercariae were still immature and all the sporocysts had developed beyond the early irregular stages of growth. Such rapid growth and development starting immediately after the migration is probably associated with a greatly improved food supply for the daughter sporocysts in their new location as compared with that obtained while still crowded together in the mother sporocyst mass.

The method of growth of the daughter sporocysts of *P. muris* is so peculiar that it seems almost inconceivable that similar stages should not have been frequently seen if this type of development is at all common in the plagiorchiids. The thickenings at the ends of the earliest stages after attachment, the formation of projections from these ends and the bizarre shapes that are assumed are all very striking and should have attracted the attention of observers. We have found, however, only a few scattered observations that suggest a similar development. Among the earliest workers, de Filippi (1857, Pl. I, Fig. VIII) showed a young sporocyst inside its paletot divided into two parts by a narrow constriction. "Suckers" at the ends of immature daughter sporocysts of this group have been reported from time to time and the figures of Biehringer (1885, Tab. 1, Figs. 1, 11, 13, and 14) show that these so-called suckers for *C. armata* are merely growth areas with invaginations at their centers like those found at the ends of the very immature daughter sporocysts of *P. muris*. This author (Fig. 12) also showed a slight protrusion at the center of the growth area of one of these sporocysts which resembles our figure 18. Daughter sporocysts with projections or with irregular shapes have also been noted (Looss, 1896, Pl. XVI, Fig. 178; Harper, 1929). In fact, such findings have been, perhaps,

the chief basis for the views held by certain authors that the daughter sporocysts of the xiphidiocercariae commonly reproduce by fission and budding (Wesenberg-Lund, 1934).

There is very little information available on the germ cell cycle of the PLAGIORCHIOIDEA. The miracidia in this group are very small and in the few cases where they have been carefully studied were found to contain only a very few germ cells, as was clearly shown by Talbot (1933) and Walker (1939). These authors also studied very immature mother sporocysts and their descriptions indicate an early rapid multiplication of the germ cells to form large numbers of daughter sporocyst embryos. Our observations on *P. muris* add nothing on this stage. We have found nothing in the literature on the germ cell cycle in the development of the daughter sporocysts except the work of Brooks (1930). This author merely stated that "germinal multiplication is by the formation of germ masses." According to his view these germ masses break up into single cells, the "ex-components," each of which forms a cercarial embryo.

Our studies give some information on the germ cell cycle in the daughter sporocysts of *P. muris*. In the youngest embryos we observed there had already been a considerable multiplication of germ cells and from these cells cercarial embryos soon begin to develop. This type of development of new cercarial embryos from individual germ cells seems to continue throughout the early stages. But later a definite, discrete germ mass appears. Perhaps this develops from one of the separate germ cells, while all the others go on to cercarial embryos. Unfortunately, our observations are not at all clear on this point. At any rate, by the time the daughter sporocysts are ready to migrate from the mother each contains a single discrete, rather large germ mass surrounded by its own membrane, and no separate single germ cells are present in the body cavity. The smallest free bodies found at this stage are multicellular cercarial embryos that have the same size and structure as the largest multicellular components of the germ mass. From repeated examinations of the contents of daughter sporocysts at all stages, we conclude that in each a single germ mass persists all through development and that no free germ cells are present.

As noted above, these germ masses are still present in very old daughter sporocysts in old snails that would soon die from old age. Such sporocysts while not as crowded with developing cercariae as the younger ones, still contain numbers of mature and almost mature cercariae and all developmental stages including the very smallest embryos. The conclusion seems inescapable that after the formation of the germ mass, the only method of production of new cercarial embryos is by the splitting off of its largest multicellular components. We interpret this as a special type of polyembryony like that described for the strigeids (Cort and Olivier, 1941).

One of the most striking things in the development of *P. muris* is the long life possible for the daughter sporocysts and the very large number of cercariae that a single infection can produce. In fact, enormous cercarial production seems to be characteristic of the PLAGIORCHIOIDEA (Cort, 1922; Dubois, 1929). In the development of *P. muris* in the snail intermediate host the daughter sporocysts that migrate from a single mother sporocyst mass begin to give off mature cercariae at about the same time, perhaps in a month to six weeks after the snail is infected. It seems probable that the production of cercariae continues until the infection is terminated by the death of the snail. Therefore, in an infection that entered a very young juve-

nile snail, cercariae might begin to escape by the end of July and would be produced in increasing numbers until activity ceased with cold weather. As soon as the temperature warmed up sufficiently the next spring, cercarial production would begin again and would last until the natural death of the snail in the late summer or fall. It seems probable that in such infections each daughter sporocyst would produce hundreds of cercariae throughout its long life and that the total number that developed from a single miracidium might reach almost a million. Such an enormous production of cercariae appears to be achieved partly by a considerable multiplication of the few germ cells set aside during the development of the miracidium to form the several hundred daughter sporocysts produced by a single mother. Most important, however, is the reproductive ability of the large germ masses which develop in the daughter sporocysts before they migrate from the mother and which, by a special type of polyembryony, produce a constant stream of cercarial embryos throughout the whole life of the infection.

In addition to this extraordinary production of cercariae, which it seems to share with other plagiorchiids, *P. muris* has other special characteristics that appear to account for its great success as a parasite. Its larval stages in the snail are so located and their habits are such that they do not seem to produce serious injury to their host. Certainly, there is no evidence of an increase in the death rate of the snails due to the presence of this species such as has been found in certain other types of larval trematode infections. In addition, the metacercariae can develop not only in a variety of aquatic insect larvae, but, also, precociously inside the sporocysts in the first intermediate host, making possible infection of animals that feed on these snails. Finally, a lack of specificity in definitive host relations makes possible the development of the adult in a wide variety of both birds and mammals.

SUMMARY

During the summer of 1941 large numbers of juveniles and adults of the common beach snail, *Stagnicola emarginata angulata* (Sowerby) were examined from a beach on the shore of Burt Lake in northern Michigan, where there was known to be a high incidence of the larval stages of *Plagiorchis muris*. A considerable series of late stages of mother sporocysts was found, especially in juvenile snails. They are oval, irregularly disc-shaped masses, 0.5 to 1.7 mm in largest diameter, which are composed almost entirely of daughter sporocyst embryos (about 300 to 500 in each). These mother sporocysts are firmly attached to the outside of the snail's intestine, and may occur anywhere along its whole length. The matrix of the mother surrounding the daughter sporocyst embryos consists of irregular cells which form an outer layer around the daughter sporocyst embryos. This outer layer, the paletot of earlier workers, persists throughout the whole life of the daughter sporocysts. The largest daughter sporocysts still found in the mother sporocyst masses are elongate and mobile, and each contains cercarial embryos of different ages, and a single discrete germ mass. When located on the parts of the intestine between the foot and digestive gland they break out of the mother sporocyst, all at about the same time, and migrate along the ducts and vessels to the walls of various organs and to all parts of the digestive gland of the snail. When the mother sporocysts are on the surface of the gland the migration of the daughters is much more limited and there is a tendency for them to develop without leaving the mother. When the daughter

sporocysts cease migrating they become firmly attached, thicken, and soon appear crowded with embryos. Soon, thickened areas are formed at their ends, which frequently have invaginations at their centers making them look like suckers. From these "areas of growth" protrusions push out. Further growth of these protrusions and their occupation by cercarial embryos produce a variety of irregularly shaped sporocysts, each enclosed in its paletot. Later, with increasing size of the sporocyst wall and rapid growth and production of cercarial embryos, the shapes of the daughter sporocysts become less irregular. Growth in size of the sporocysts continues after the escape of the first mature cercariae, and the paletot becomes pigmented after maturity is reached. The germ mass persists throughout the whole life of the daughter sporocyst, and produces a constant stream of cercarial embryos by a process that has been interpreted as a special type of polyembryony like that found in the strigeids. Even in the very oldest, most heavily pigmented sporocysts from old snails there is no sign of exhaustion of cercarial production. Infections of *P. muris* enter the snail host both in its juvenile and adult stages and probably produce cercariae actively until the death of the host. Because of their position along the ducts and vessels and on account of their inactivity, the daughter sporocysts do not appear to produce serious injury to their host. It is suggested that the great success of *P. muris* as a parasite is due to its extraordinary power of cercarial production, the absence of serious injury to the snail by its larval stages, and the lack of specificity of its metacercarial and adult stages.

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REPORT ON A COLLECTION OF BRANCHIOBDELLIDS

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The writer has had the opportunity to examine an interesting collection of branchiobdellids which were received from G. K. Goellner of the University of Michigan. In the collection, two new forms were encountered, and several new records of previously described forms have been added to our knowledge of the distribution of this interesting group of worms.

Cambarincola chirocephala Ellis, 1919

Records: Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner; and James River, Gallow, Missouri, Coll. Creaser and Williamson.

Cambarincola macrocephela n. sp.
(Figs. 1 and 2)

Description: Cambarincola: Worm cylindrical, head large, expanded so that it is distinctly wider than anterior body segments. Head separated by deep constriction from Segment I. Dorsal lip with four finger-like projections, ventral lip entire. Body segments increasing in width from I to VII, posteriorly decreasing to the terminal sucker. Major annulations of body segments not elevated over minor annulations. Ventral and dorsal jaws similar, appearing as large triangular blocks of chitin terminating in sharp tooth. Without lateral teeth but margin uneven. Width of jaws at base 400 μ. Pharyngeal diverticula four, two dorsal and two ventral. Alimentary canal in medial portion straight, with large sacculation in segments II and III and small sacculations in segments IV, V, VI and VII. Posterior to segment VII, narrows rapidly to anus in dorsal portion of segment X. Testes in pairs in segments V and VI. Vasa deferentia joining atrium in VI; with accessory sperm tube. Ovaries in segment VII. Length of mature worm 2.0 to 4.0 mm.

Locality: Polecat Creek, Northern Teton County, Wyoming, August 16, 1941. Coll. Robert C. Brown.

Host: Astacus gambellii.

Holotype specimen: U. S. Nat. Mus. Coll. No. 20598.

This species differs from all known members of *Cambarincola* in the type of jaws.

Cambarincola macrondonta Ellis, 1912

Records: Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner; and State Hatchery Ponds, Pratt, Pratt County, Kansas, October, 1939, Coll. Robert C. Brown.

Cambarincola philadelphica (Leidy, 1851)

Records: Muddy Creek, La Monte, Missouri, September 2, 1930, Coll. E. P. Creaser; and Reed Creek, Wortle County, Virginia, May 17, 1931, Coll. C. L. Hubbs and E. P. Creaser.

Cambarincola vitrea Ellis, 1919

Records: Cheyenne, Wyoming, September 18, 1941, Coll. James C. Simpson; and Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner.

Pterodrilus distichus Moore, 1895

Record: Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner.

Xironodrilus appalachius n. sp.
(Figs. 3 and 4)

Description: Xironodrilus: Body rather elongate, and distinctly depressed. Width of head approximately equal to segment I. Body segments increasing regularly in width from segment

Received for publication, March 23, 1942.

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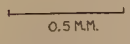

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FIG. 1. Dorsal view of *Cambarincola macrocephela* n. sp.
FIG. 2. Dorsal jaw of *Cambarincola macrocephela* n. sp.
FIG. 3. Dorsal view of *Xironodrilus appalachius* n. sp.
FIG. 4. Dorsal jaw of *Xironodrilus appalachius* n. sp.
FIG. 5. Dorsal jaw of *Xironodrilus dentatus* Goodnight, 1940.

I to VII, posteriorly decreasing to caudal sucker. Small accessory suckers present near lateral margins of ventral surfaces of segments VIII and IX. Segments I to IX distinct, major annulations not elevated over minor elevations. Upper and lower lips entire, with small median emarginations. Tooth formula 3–3. Median tooth longer than lateral teeth. Major pharyngeal diverticula two in number, one dorsal and one ventral. Alimentary canal passing through body near medial axis, somewhat expanded in segment I, strongly sacculated in segments II to V, narrowing in segment VI, somewhat enlarged in segments VII and VIII, narrowing rapidly from segment VIII to anal opening on dorsal surface of segment X. Testes present in segments V and VI, vasa deferentia joining atrium segment VI. No accessory sperm tube present. Spermatheca

in segment V, simple. Each anterior nephridia opening to outside through separate pores on dorsal surface of segment III. Ovaries present in segment VII. Length of mature worm approximately 3 mm. Width of upper and lower jaw at the base 60 μ.

Locality: Clingman's Dome, Great Smoky Mountains National Park, September 9, 1937. Coll. A. R. Cahn.

Host: Cambarus sp.

Holotype specimen: U. S. Nat. Mus. Coll. No. 20596.

This species shows its closest affinities to *Xironodrilus pulcherrimus* (Moore, 1894), but differs in having the median tooth longer than the lateral ones, whereas in *pulcherrimus* it is shorter.

Xironodrilus dentatus Goodnight, 1940
(Fig. 5)

Record: James River, Gallow, Missouri, Coll. Creaser and Williamson.

Additional material found for study, leads the writer to believe that this name should be elevated to specific rank instead of subspecific rank as originally defined. In all cases, the tooth formula was much greater than in the typical *pulcherrimus*. The upper jaw is shown in Fig. 5. A holotype specimen has been deposited in the U. S. National Museum Collection, No. 20595.

ACANTHOCEPHALA OF THE GENUS *CORYNOSOMA* FROM THE CALIFORNIA SEA-LION

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In a study of four lots of Acanthocephala from the California sea-lion, *Zalophus californianus*, two species of the genus *Corynosoma* Lühe, 1904, have been found. One of these has apparently been previously described from Japanese hosts; the other has been apparently heretofore unrecognized in the literature.

In 1935 Harada described *Corynosoma ambispinigerum* from the small intestine of *Phoca* sp. from North Pacific waters off Japan. In an appendix to his paper Harada stated that *C. ambispinigerum* was a synonym of *C. osmeri* which was described by Fujita in 1921 from immature forms collected from the peritoneal membrane of *Osmerus lanceolatus* taken at Mukawa, Hokkaido, Japan. Harada recognized a discrepancy between Fujita's published description and his own observations on some of the original specimens of *C. osmeri*. According to Fujita the proboscis hooks of *C. osmeri* are arranged "28 in each 19–12 circular" row, whereas Harada by his own observations on some of the same material found 18 longitudinal rows of hooks with 10–11 in each row.

Two of the lots from the California sea-lion contained five specimens of an acanthocephalan which is apparently identical with the specimens that Harada named *C. ambispinigerum*. Accepting Harada's conclusion that *C. ambispinigerum* is not a separate species, the valid name for the specimens under consideration here becomes *Corynosoma osmeri* Fujita, 1921.

The following is a description of *C. osmeri* based upon study of two females

Received for publication, June 10, 1942.

* The writer wishes to extend his thanks to Dr. Carlton M. Herman and Dr. Emmett W. Price who have made the collections in this report available for study.

(apparently neither fully mature) and three males which adds some details omitted by both Harada and Fujita.

Corynosoma osmeri Fujita, 1921
(Figs. 2, 3, 5, 6, 9)

Body approximately 6 mm long divided into two regions: 1) anterior swollen, bulbous region, 2) posterior elongated trunk (Fig. 9). Body spination in two fields: one covering anterior bulbous region and extending but a short distance on ventral surface of trunk; the other around genital pore, which is arranged in about six quincuncial rows in males, and only a few scattered spines in females. Spines on bulbous part of body 28–52 μ long and 5–20 μ in maximum width. Trunk spines 24–44 μ long and 6–8 μ in maximum width. Genital pore subterminal, slightly dorsal.

Bulbous end of body bent ventrally so that proboscis protrudes at obtuse angle from longitudinal axis of body (Fig. 9), as characteristic for most species of *Corynosoma*. Proboscis 651–686 μ long in females, no measurements on males; swollen just posterior to mid-region (Fig. 5); vesicle of unknown origin or function present in swollen area; 176–180 μ wide at anterior end in females, 211 μ in male; 237–246 μ at swollen area in females, no measurements on males; armed with approximately 18 longitudinal rows of hooks, each row with 11 hooks in both sexes. Measurements, in microns, of proboscis hooks as follows:

		Length	Width (at angle)	Length of root
Anterior hooks	♀	44–78	13–16	56–64
	♂	68–72	16–22	44
Conspicuous hooks on swollen area	♀	72–76	28–36	82–86
	♂	64–80	26–28	76
Basal hooks	♀	40–42	6–16	40–60
	♂	no measurements		

Anterior hooks as well as thickened hooks near mid-region of proboscis have strongly recurved roots (Fig. 2). Hooks at base of proboscis have inverted Y-shaped root (Fig. 3).

Proboscis sheath double-walled, no accurate measurements on length and width possible. Lemnisci probably shorter than proboscis sheath.

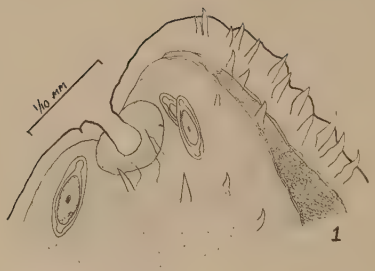
Testes two, situated nearly side by side in anterior half of body near posterior end of proboscis sheath. Cement glands six in number, elongated, clavate in shape immediately following the testes (Fig. 9). Eggs 52–78 μ long and 16–18 μ wide with polar outpocketings (Fig. 6).

In describing the proboscis hooks of *C. ambispinigerum* Harada made no mention of conspicuously thickened or heavy hooks on the swollen area of the proboscis, nor in his figure of the proboscis of this animal did he indicate that any such outstanding hooks were present. Similarly Fujita described no prominent hooks on the proboscis of *C. osmeri*. In the specimens at hand the hooks on the swollen area of the proboscis are conspicuous.

The second species of *Corynosoma* present in the collections from *Zalophus californianus* apparently has been heretofore unrecognized in the literature. For this species the name *Corynosoma obtuscens* is proposed.

Corynosoma obtuscens n. sp.
(Figs. 1, 4, 7, 8)

Specific diagnosis: Body small, almost in form of inverted L, anterior end swollen into bulb and bent ventrally; 2.2–3.0 mm long in females, 2.0–2.8 mm in males. Maximum thickness (dorsoventral) of posterior body 0.4–0.7 mm in females, 0.3–0.5 in males.



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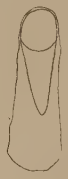

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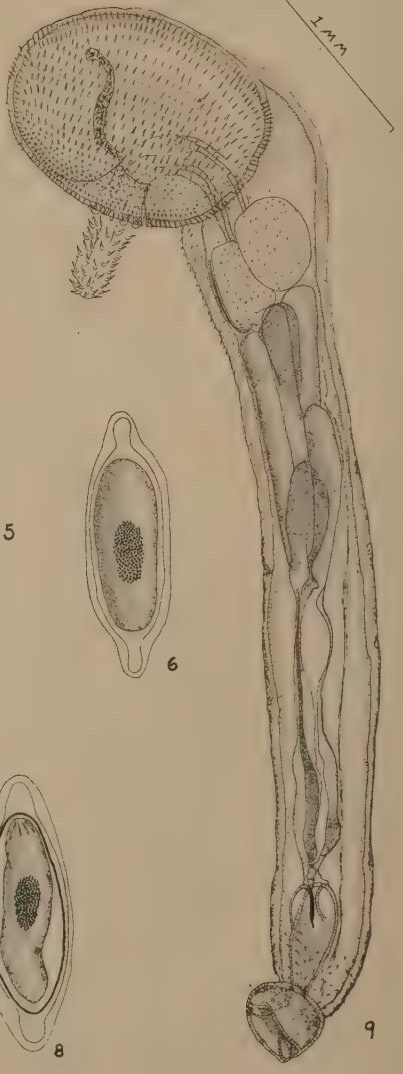

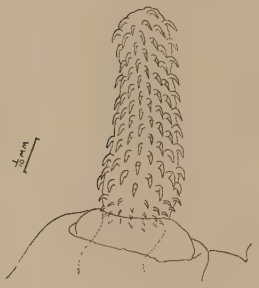

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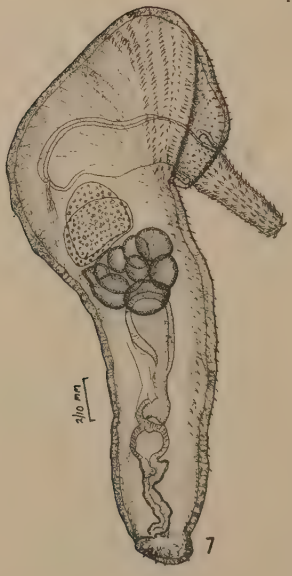

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Anterior bulbous part of body on both dorsal and ventral surfaces closely set with spines; trunk with spines only on ventral surface, in females extending all the way to genital pore (Fig. 1), in males most of way but separated from genital spines by area devoid of armature (Fig. 7). Spines on fore part of body 28–52 μ long in females, 32–50 μ in males; spines on posterior trunk of females 28–46 μ long, of males (genital spines excepted) 30–40 μ long. Male genital spines set in about six rows, approximately 30 in number and 29–48 μ long, 12–20 μ in maximum width.

Proboscis bent at obtuse angle from longitudinal axis of body, swollen near base (Figs. 4 and 7), 528–616 μ long in females, about 546 μ in males. Width of proboscis at anterior end 116–156 μ in females, 104–140 μ in males; at swollen region 180–204 μ in females, 144–180 μ in males; at base 160–188 μ in females, 144–160 μ in males.

Proboscis hooks in 16–19 longitudinal rows in females, 17–18 in males, with 12–13 hooks in each row (Fig. 4). Measurements, in microns, of proboscis hooks as follows:

	Length				Width			
	Anterior (from tip to swollen area)		Posterior (below swollen area)		Anterior (from tip to swollen area)		Posterior (below swollen area)	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
♀	46–56	44–56	28–40	16–32	8–18	8–18	4–10	6–12
♂	36–50	40–56	28–30	28	8–16	8–16	6–9	6–8

Proboscis sheath double-walled, no measurements possible. Lemnisci short, flattened and extending about three quarters length of proboscis sheath. Main longitudinal canals of lacunar system lateral.

Testes two, located well forward, side by side, immediately behind proboscis sheath. Cement glands 6 (8?), pyriform, grouped in a cluster closely behind testes. Genital opening subterminal, slightly dorsal in position in both sexes. In female strong sphincter muscle guarding genital opening (Fig. 1). Eggs 68–90 μ in length and 20–28 μ in width with polar outpocketings (Fig. 8).

Host: Zalophus californianus, the California sea-lion.

Habitat: Intestine.

Locality: Zoological Gardens, San Diego, Calif.

Types: Holotype male (DL 121.23) and allotype female (DL 121.24) in collection of writer. Paratypes in collection of writer, H. J. Van Cleave in Urbana, Illinois, and deposited in the U. S. Nat. Mus. No. 36843.

Many species of the genus *Corynosoma* have essentially the same proboscis hook formula. If the factors of natural variability and difficulties of accurate observation are taken into account the pattern of the proboscis hooks becomes a poor criterion for the separation of species in this genus, unless the pattern is of such a character as to be distinctive and is beyond the expected variation due naturally or to faulty observation.

C. obtuscens differs significantly from five species as to the kind, number and arrangement of the proboscis hooks. In *C. semerme* (Forssell, 1904) there are

FIG. 1. Lateral view of posterior tip of female *Corynosoma obtuscens.*
FIG. 2. Frontal view of anterior proboscis hook of *Corynosoma osmeri.*
FIG. 3. Hook from basal portion of proboscis of *C. osmeri.*
FIG. 4. Proboscis of *C. obtuscens.*
FIG. 5. Proboscis of *C. osmeri.*
FIG. 6. Egg of *C. osmeri.*
FIG. 7. Male of *C. obtuscens.*
FIG. 8. Egg of *C. obtuscens.*
FIG. 9. Male of *C. osmeri.*
All figures drawn with the aid of a camera lucida except Figs. 2, 3, 6 and 8.

22–24 longitudinal rows each with 12–13 hooks (Meyer, 1932) and according to Lühe (1911) there are 24–26 longitudinal rows. In *C. reductum* (von Linstow, 1905) there are 24 rows of 10–11 hooks; in *C. hamanni* (von Linstow, 1892) 28 rows of 10 hooks. *C. obtuscens* may be distinguished from *C. turbidum* Van Cleave, 1937 as well as *C. osmeri* Fujita, 1921 by the fact that the latter two species have large conspicuous hooks arming the swollen region of the proboscis.

The remaining species of *Corynosoma* all have hook patterns of approximately eighteen longitudinal rows with nine to fourteen hooks per row. *C. obtuscens* may be distinguished from *C. strumosum* (Rudolphi, 1802), *C. pyriforme* (Bremser, 1824), *C. bullosum* (von Linstow, 1892), *C. tunitae* (Weiss, 1914), *C. osmeri* Fujita, 1921 (syn. *C. ambispinigerum* Harada, 1935), *C. australe* Johnston, 1937, *C. antarcticum* (Rennie, 1907) (syn. *C. sipho* Railliet and Henry, 1907, according to Johnston and Best, 1937), *C. peposacae* (Porta, 1914), *C. phalacrocoracis* Yamaguti, 1939, and *C. mergi* Lundström, 1941 with respect to the kind and distribution of body spines.

C. obtuscens differs from *C. constrictum* Van Cleave, 1918 in that there are no body constrictions such as have been described for the latter.

Yamaguti (1935) records a species of *Corynosoma* which, due to insufficient material, has not been specifically identified. From the fragmentary information given it appears that *C. obtuscens* is distinct from *Corynosoma* sp. of Yamaguti on the basis of hook size and body spination. The hooks of *Corynosoma* sp. are longer and the body spines on the anterior end are in two circular bands.

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THE RATE OF GROWTH OF *ANOPHELES QUADRIMACULATUS* IN RELATION TO TEMPERATURE

HERBERT S. HURLBUT*

Insects in general exhibit an increased rate of growth with a rise in temperature. In particular species the relation of temperature to growth is sufficiently constant to furnish a basis for predicting growth rates at known temperatures. This study was undertaken in order to provide a working basis for predicting the growth rates of *Anopheles quadrimaculatus* under the varying temperature conditions found in nature.

LABORATORY OBSERVATIONS WITH CONSTANT TEMPERATURES

A domesticated strain of *A. quadrimaculatus* was reared at temperatures of 82° ± 1°, 74° ± 2°, and 64° ± 1° F. Rearing was unsuccessful at 53° ± 1° F, but eggs and pupae completed their development. Food, consisting of pulverized commercial dog food, was supplied according to the method employed routinely for the stock colony (Crowell, 1940). The observations have been grouped under three headings, the egg stage, the pupal stage, and the complete cycle from egg to adult. In order to facilitate comparison between different temperatures, temperature summation has been employed in the calculation of a time-temperature value, using 50° F as the developmental zero. The data suggest that this approximates the true zero. The average of the time-temperature values at different temperatures has been utilized as a thermal constant. If one assumes that the rate of development is directly proportional to the temperature, the relationship may be expressed by the formula, $a(b-c) = k$, in which a is time, b is temperature, c is the temperature below which development does not occur, and k is the thermal constant. The degree of conformity to this relationship may be judged by the variability of the observed time-temperature values.

Egg Stage

Eggs were collected within one hour after deposition and subjected immediately to the experimental conditions. Counts of larvae were made at hourly intervals throughout the period of hatching. The data are summarized in Table 1.

TABLE 1.—*Egg stage*—Anopheles quadrimaculatus

Time in hours			Temperature degree F.	Total degree-hours for mean period. Develop. zero 50° F	Total eggs hatching	Per cent hatching
Mean	Min.	Max.				
38.1	37	42	82 ± 1	1219	129	82
38.7	37	40	82 ± 1	1238	176	..
54.1	53	60	74 ± 2	1298	116	87
57.9	56	61	74 ± 2	1390	107	..
108.8	104	112	64 ± 1	1509	152	76
357.9	324	420	53 ± 1	1074	135	72
368.1	360	384	53 ± 1	1104	119	83

If one applies the method of thermal summation, assuming a developmental zero of 50° F, the degree-hour value is seen to vary from 1074 to 1509. More closely

Received for publication, May 19, 1942.

*From the Health and Safety Department, Tennessee Valley Authority, Wilson Dam, Alabama.

controlled low temperatures might have produced less variation in this figure since a change of 0.5 to 1.0 degree would have given values approximating those found for the higher temperatures. However, if the figures are averaged as they stand, a value is obtained which it is believed is of practical use in estimating the duration of the egg stage at any particular temperature within the range indicated. At extremes of temperature it is well known that the method of thermal summation becomes unreliable. Fig. 1 shows graphically the time-temperature curve obtained

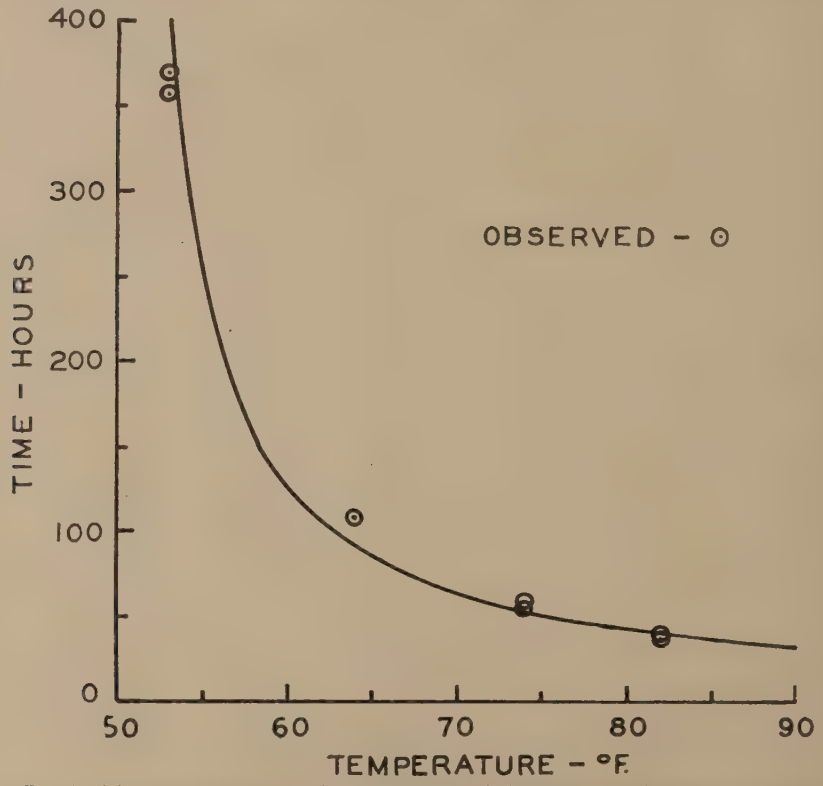

FIG. 1. Time-temperature curve for the egg stage of *Anopheles quadrimaculatus* at constant temperatures, based on the formula: hours (degrees F–50) = 1262. The observed points are indicated.

by using this value, together with the points determined experimentally. The formula employed in constructing the curve is as follows: Hours (degrees F $-50) = 1262$.

Pupal Stage

The duration of the pupal stage was observed at 82° ± 1°, 74° ± 2°, and 53° ± 1° F. The data are presented in Table 2. Again applying the method of thermal summation with an assumed developmental zero of 50° F, the degree-hour value is seen to vary from 930 to 1262. The number of pupae and the number of temperatures are probably too small for accurate determination. However, a trend

TABLE 2.—*Pupal stage*—Anopheles quadrimaculatus

Time in hours			Water temperature degree F	Total degree-hours for mean period. Assumed developmental zero—50° F	Total adults emerging	Per cent emerging
Mean	Min.	Max.				
38.1	37	40	82 ± 1	1219	14	100
52.6	49	55	74 ± 2	1262	11	100
309.9	303	315	53 ± 1	930	11	84

similar to that observed for the incubation period is seen here also. It is of interest to note that the hour-degree value is almost identical to that of the egg stage.

Egg to Adult

The duration of development from egg to adult appeared to be directly proportional to the temperature, at constant temperatures of 82° F and 74° F. The departure at 64° F may have been due to an insufficient number of specimens reared to maturity. The data are presented in Table 3. Although embryonic development

TABLE 3.—*Egg to adult, constant temperature*—Anopheles quadrimaculatus

Time in days egg to adult			Water temperature degree F	Total degree-days for mean period. Dev. zero 50° F	Total adults emerging	Per cent reared
Mean	Min.	Max.				
11.70	11	14	82 ± 1	374	54	42
12.08	11	14	82 ± 1	386	38	22
16.18	15	18	74 ± 2	388	82	70
16.53	15	18	74 ± 2	396	94	88
36.85	28	43	64 ± 1	516	36	24

was completed and hatching took place at 53° F, the larvae failed to develop further, all dying within a few days after hatching, without perceptible growth. The degree-day value secured by averaging the value for the individual groups, excluding the one for 64° F, is 386.

GROWTH RATES AT OUTDOOR TEMPERATURES

Larvae were reared in small cages made of bolting silk (Fig. 2) in a natural breeding place. The cages were 10 inches square by six inches deep, with a top of

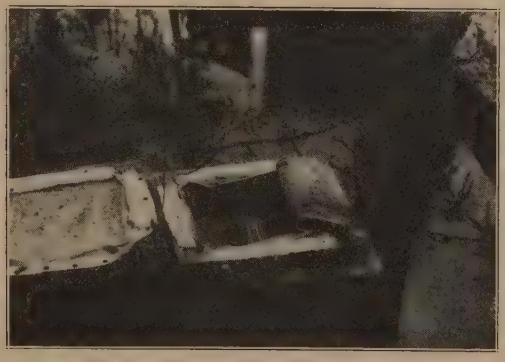

FIG. 2. Equipment for outdoor rearing of *Anopheles quadrimaculatus*. One rearing cage is opened showing thermometer.

bolting cloth closed by a zipper fastener. They were fastened to a light wooden frame which fitted into a square opening in a raft made of cypress. Large, burned-out electric bulbs were utilized as floats. The cages were so placed that the water depth in them was about four inches, leaving two inches between the water and the top of the cage. The raft floated freely so the water level inside the cage remained constant. A maximum and minimum self-registering thermometer was suspended horizontally inside one of the cages at about $\frac{1}{4}$ inch below the water surface. Eggs from captive wild females were introduced into a cage about 12 to 18 hours after oviposition. The number of specimens in each stage was counted daily and the maximum and minimum temperatures recorded. The naturally occurring food was supplemented by liberal amounts of the pulverized commercial dog food added daily. An abundant growth of filamentous algae was generally present. In the spring *Spirogyra* and *Ulothrix* comprised most of the growth, but during the summer these were replaced naturally by *Oscillatoria.*

The data from these observations at outdoor temperatures are recorded in Table 4. The first generation was reared from eggs deposited in the spring by overwinter-

TABLE 4.—*Egg to adult, outdoor temperatures*—Anopheles quadrimaculatus

Date, 1941	Time in days egg to adult			Water temperature degree F			Total degree-days for mean period develop. zero 50° F	Total adults emerging	Per cent reared out
	Mean	Min.	Max.	Mean	Min.	Max.			
Mar. 23–Apr. 30	33.4 ± 0.24*	30	38	63.9	45	79	465	75	74
Apr. 27–May 20†	21.0 ± 0.11	18	23	74.4	63	88	514	80	83
May 19–June 4	14.6 ± 0.13	12	16	82.1	73	90	469	46	51
June 7–June 27	18.0 ± 0.23	15	20	83.6	77	92	606	36	47
June 26–July 13	15.6 ± 0.06	14	17	87.2	80	95	580	119	42
July 29–Aug. 16	15.3 ± 0.23	12	18	88.3	84	94	586	52	32
Aug. 30–Sept. 18	15.1 ± 0.12	12	19	85.0	80	91	528	128	69
Sept. 14–Oct. 4	15.4 ± 0.14	12	20	81.8	76	90	490	194	89

* Standard error.
† Domesticated strain.

ing females. The females had been collected in early November and were kept in a cave throughout the winter. Ten generations were reared in series from March through October. The generation of July 13 to 29 is omitted from the table because the pupae were largely destroyed by predators, and that of October 4 to 27 because of insufficient data. The results, however, were in general agreement with the observations presented. Each generation was derived from captive wild females with the exception of the April 27 to May 20 group which was from the domesticated strain.

A mean temperature for each generational period was calculated by averaging the maximum and minimum daily temperatures. Hourly readings in most instances gave a mean in fairly close agreement with the daily mean derived from the maximum and minimum, though this may well be a source of error in determining the time-temperature value. The product of the mean temperature minus 50 and the average number of days gave a degree-day value which varied from 465 to 606, the

average being 530. A time-temperature curve based on this value is given in Fig. 3, the points being determined by the formula: days (degrees F – 50) = 530. While considerable variation was observed, it would appear that temperature summation based on maximum and minimum daily temperature does provide a working basis for predicting the duration of the life cycle of this species under the varying temperatures occurring in nature. Thus the total degree-days required in early spring

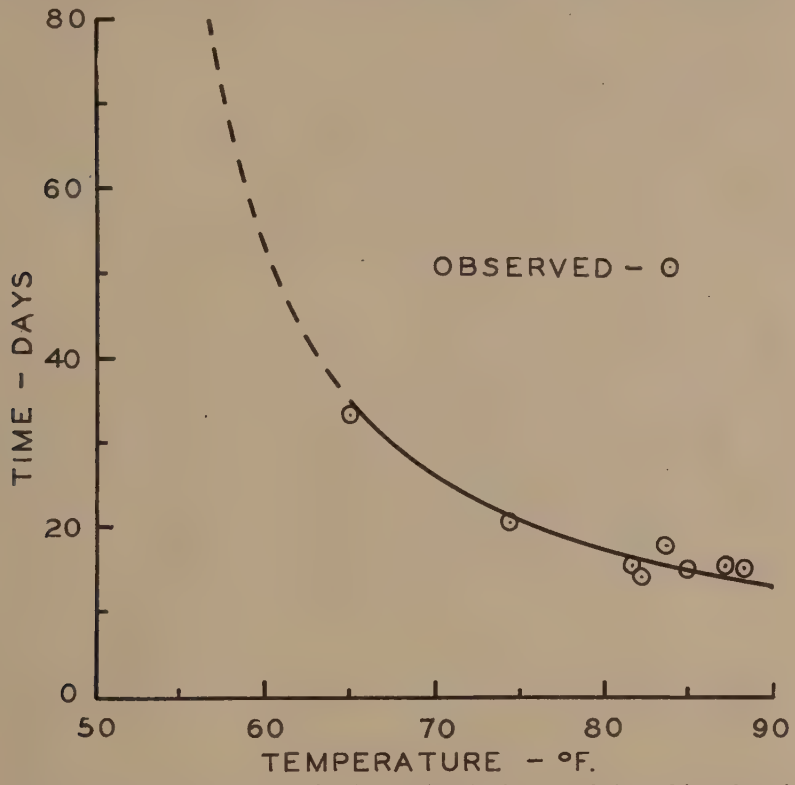

FIG. 3. Time-temperature curve for the complete development of *A. quadrimaculatus* from egg to adult at outdoor temperatures, based on the formula: days (degrees F–50) = 530. The observed points are indicated.

when the range was from 45° to 79° F approximates the value found for midsummer when there was but slight variation from day to day in the maximum and minimum temperature. Undoubtedly there are important factors other than temperature which are involved, particularly food. An attempt was made to hold this factor as nearly constant as possible. Obviously this could not be done within strictly narrow limits under semi-natural conditions, or even under laboratory conditions, in the present state of our knowledge of the nutritional requirements of this species.

Attention is called to the fact that the time-temperature value at constant temperatures is much smaller than at outdoor temperatures. The method of obtaining

the mean temperature might account for this in part though it is difficult to explain such a wide discrepancy on this basis.

OBSERVATIONS IN THE NATURAL HABITAT

While a detailed study under strictly natural conditions has not been made, a few available observations can be recorded. On several occasions 4th instar larvae were found to be present in a natural breeding place, 6 to 8 days after refilling, subsequent to dewatering of the area. This was during July, August and September when it was observed that the duration of the developmental period did not vary significantly under experimental conditions. In another study (Hinman, 1939) it has been observed that the larval instars of *A. quadrimaculatus* tend to show successive peaks of abundance in its natural habitat. Thus, a peak for 1st instar larvae was followed after 7 to 9 days by one for 4th instar larvae. From the experimental data, the mean duration of larval life during the summer months is calculated to be about 12 days. If one deducts 3 to 4 days for the incomplete 1st and 4th instar periods, the result is in accord with the observations in the natural habitat.

PRACTICAL APPLICATION

The time of initiation of significant *A. quadrimaculatus* breeding in the spring is of vital concern to malaria control workers. In the Tennessee Valley, it is believed that this species passes the winter primarily as inseminated adult females (Hinman and Hurlbut, 1940). They take blood and deposit their eggs in the early spring. As already indicated, the 1st generation reared at outdoor temperatures in this study was derived from overwintering females. Eggs were deposited on March 23, and the generation was completed on April 25. A 2nd generation started on April 27 was completed May 20. This latter date approximated the first appearance of adult *A. quadrimaculatus* in significant numbers in nature. Typically, the population density of this mosquito in the lower Tennessee Valley is characterized by a marked increase sometime during the first three weeks of May. This ordinarily marks the appearance of the species in significant numbers. On the basis of these observations it is suggested that this first marked increase in numbers in the spring is indicative of the appearance of the 2nd generation. This would assume that there is a more or less synchronous development of the 1st and 2nd generations. Conditions in nature appear to favor this, since low temperatures at the beginning of the season tend to delay development of eggs deposited then so that those deposited later, at somewhat higher temperatures, actually are not very far behind. Thus groups started at widely separated dates early in the season would mature at approximately the same time. Observations made on the hatching of eggs at this season tend to support this assumption. It was also observed that larvae hatching before daily average surface water temperatures began to consistently exceed 50° F did not survive well.

The application of the method of temperature summation should thus provide a basis for forecasting the seasonal reappearance of this species in significant numbers. Starting with the date when the average daily surface water temperature in a representative breeding place begins to exceed consistently 50° F, the number of degrees in excess of 50 should be summed daily until the degree-day value of 530 is reached. This would indicate that the season had advanced sufficiently to permit

one generation to reach maturity. Repetition of the process would furnish a guide to the progress of the 2nd generation, against which the initial control measures of the season should probably be directed.

Making due allowance for ovarian development, it is estimated from the data presented that *A. quadrimaculatus* may pass through 9 or 10 generations annually in northern Alabama. From field observations on the adults of this species, Boyd (1930) has estimated 7 or 8 annual generations in North Carolina, the latitude of which is slightly further north.

In the application of control measures the minimum duration of the life stages should be considered. For measures effective against all stages of development the degree-day value of 441 has been calculated from the data provided in Table 4. To determine the approximate minimum time from egg to adult at a known average temperature, the following formula may be used: days (degrees F – 50) = 441. For measures effective against the larval stages only, the degree-day value of 305 has been calculated. To determine the minimum duration of the combined larval stages this value should be substituted in the above formula.

SUMMARY

The rate of development of *Anopheles quadrimaculatus* was observed at constant temperatures in the laboratory and at varying temperatures under outdoor conditions. The observations at outdoor temperatures were made continuously throughout one complete season from March through October. While the data do not support the unqualified assumption that the rate of development is directly proportional to the temperature, it is believed that the relationship is sufficiently close to be of practical value in estimating the duration of the life cycle when the temperature is known. The observations at outdoor temperatures indicate that temperature summation can be utilized as a working basis for prediction of growth rates under natural conditions.

On the basis of the data presented it is estimated that *A. quadrimaculatus* may pass through 9 or 10 generations annually in northern Alabama. The annual reappearance of adults in significant numbers during the first three weeks of May is believed to mark the emergence of the 2nd spring generation. A method is suggested for predicting the time of appearance of this generation when daily maximum and minimum water temperatures are known.

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NATURAL TRANSMISSION OF IMMUNITY AGAINST *TRICHINELLA SPIRALIS* FROM MOTHER RATS TO THEIR OFFSPRING

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It has been previously shown in this laboratory that the specific immunity developed by mother rats against infection with certain species of trypanosomes is transmitted to their offspring, the transfer of protective substance taking place largely or entirely through the immune mother's milk (1–4). In the present paper, the results are presented of a test for the transmission of immunity against a nematode parasite, *Trichinella spiralis,* from mother rats to their young, especially in respect to the route of this transfer. Rats are known to become immune to this parasite as a result of recovery from infection (5–7). The transmission of immunity from mother rats as well as from mother rabbits and hamsters to their young has also been demonstrated (8), although the precise route of such transfer is still obscure.

GENERAL METHODS

The immune mother rats were prepared by mating normal adult females which had been fed 2000 isolated living *Trichinella* larvae. Mothers infected from two to five months prior to parturition seemed able to transfer the most effective level of immunity to nurslings.

Similar quantitative methods were used in the present work as in previous studies upon experimental trichiniasis reported from this laboratory (9). The young rats were infected by mouth with 250 isolated *Trichinella* larvae suspended in nutrient broth to which 20 per cent gelatin had been added. The larvae were administered by syringe through a blunted 22-gauge hypodermic needle lowered into the retropharynx. Some animals were autopsied after five days and their intestines searched for adult worms. This was done by slitting the intestine lengthwise and agitating it in physiological salt solution. The solution was then examined with a low power microscope for the presence of adult worms. The remainder of the rats were killed after 34 days, skinned and eviscerated, and their muscle tissue digested in artificial gastric juice. Samples of the digestate were transferred to Syracuse watch glasses and the number of larvae contained in the samples determined. The number of worms in the entire carcass was then established by calculation.

RECOVERY OF ADULT TRICHINELLA FROM THE INTESTINES OF YOUNG RATS NURSING IMMUNE AND NORMAL MOTHERS

Immediately after their delivery, four of nine young of a mother rat immune to *Trichinella spiralis* were exchanged with four of eight freshly delivered young of a normal mother. Seven days later all the young were infected with 250 isolated larvae. After nursing for five days, all the young were killed, and the number of adult *Trichinella* in the intestine determined. The numbers of worms recovered from each animal are shown in Table 1.

Those young which nursed the immune mother yielded fewer adult *Trichinella* than did those which nursed the normal mother. It is particularly significant that this was true no matter whether the young had been born of the immune or of the normal mother. If the young of the normal mother were transferred at birth to the

Received for publication, May 21, 1942.

TABLE 1.—*Recovery of adult* Trichinella spiralis *from the intestine of young rats nursing a normal mother or a mother immune to* Trichinella

Young rat no.	Born of mother no.	Nursed by mother no.	No. of adult *Trichinella* recovered	Percentage recovered as adults	Average percentage recovered
1	900 (Immune)	900 (Immune)	2	0.8	3.0
2			2	0.8	
3			10	4.0	
4			12	4.8	
5			12	4.8	
6	915 (Normal)	900 (Immune)	1	0.4	1.0
7			2	0.8	
8			3	1.2	
9			4	1.6	
10	915 (Normal)	915 (Normal)	55	22.0	24.8
11			59	23.6	
12			67	26.8	
13			67	26.8	
14	900 (Immune)	915 (Normal)	28	15.2	24.4
15			55	22.0	
16			71	28.4	
17			80	32.0	

Young rats infected, when 7 days old, with 250 larvae; autopsied 5 days later.
Young exchanged at birth, before nursing own mother.

immune mother, they became as resistant as were those which had been born of this mother and which continued to nurse her. Those young transferred at birth from the immune to the normal mother were, conversely, as susceptible as were the normal mother's own young which had nursed the normal mother. Therefore, the immunity against *Trichinella spiralis* was, so far as could be determined, acquired by the young entirely after birth, through ingesting the milk of the immune mother.

RECOVERY OF LARVAL TRICHINELLA FROM THE MUSCLES OF YOUNG RATS WHICH HAD NURSED IMMUNE OR NORMAL MOTHERS

Four of eight young born of a rat immune to *Trichinella spiralis* were exchanged at birth with four of eight young born of a normal mother. When 13 days old, all

TABLE 2.—*Recovery of larval* Trichinella spiralis *from the muscles of young rats which have nursed a normal mother or a mother immune to* Trichinella

Young rat no.	Born of mother no.	Nursed by mother no.	No. larvae recovered from muscles	Average recovery
21	900 (Immune)	900 (Immune)	2,430	10,977
22			11,151	
23			12,996	
24			17,334	
25	916 (Normal)	900 (Immune)	4,788	8,770
26			5,022	
27			6,804	
28			18,468	
29	916 (Normal)	916 (Normal)	45,456	55,944
30			45,942	
31			57,650	
32			74,730	
33	900 (Immune)	916 (Normal)	41,209	51,757
34			52,689	
35			55,188	
36			57,945	

Young rats infected, when 13 days old, with 250 larvae; autopsied 34 days later.
Young exchanged at birth, before nursing own mother.

the young were infected with 250 isolated larvae. After 34 days, they were killed, skinned, and eviscerated, and their muscle digested in artificial gastric juice. The total numbers of larvae in the carcass were then estimated after careful counts upon samples of the digestate. The results are given in Table 2.

The average number of larvae recovered from the muscles of those rats which nursed the immune mother was approximately one-fifth that of the rats which nursed the normal mother. No significant difference in resistance between the young of the two mothers was apparent that could not be explained by the transmission of protective substance through the milk of the immune mother. Regardless of parent, so long as the young nursed the immune mother, they became about equally resistant. Likewise if they nursed the normal mother, they were equally susceptible, regardless of parent.

DISCUSSION

The transmission of immunity against *Trichinella spiralis* from mother rats to their young takes place in the same manner as does that against trypanosomes—that is, largely or entirely by the ingestion of the milk of the immune mother. Little or no transmission occurs while the young are in the rat uterus, by way of the placenta. Although these conclusions seem established, from the data offered, in the case of rat trichiniasis, it is, however, not necessarily true that the same route of transfer operates in the human infection with the same parasite. Most authorities feel, indeed, that in most human infections, acquired immunity is transmitted from mother to young by way of the placenta and not through the milk (10).

SUMMARY

The immunity acquired by rats after infection with *Trichinella spiralis* is transmitted to their young, the transfer occurring largely, if not entirely, through the milk of the immune mother. The young born of normal mother rats promptly become immune if permitted to nurse an immune mother rat. Conversely, the young of an immune mother are as susceptible as the young of a normal mother if they are transferred at birth to a normal mother.

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STUDIES ON THE BIOLOGY OF *ANOPHELES WALKERI* THEOBALD (DIPTERA: CULICIDAE)*

HAROLD T. PETERS

Anopheles walkeri Theobald is one of four anopheline mosquitoes found in Minnesota. While widely distributed in the state it has been considered rare, but a brief survey in 1939 (Daggy, Muegge and Riley, 1941) and the present studies in 1940 and 1941 have shown it to be very common in the Mississippi River Valley in the southeastern portion of this state.

Experiments of Matheson, Boyd and Stratman-Thomas (1933) demonstrated that *Anopheles walkeri* was an efficient transmitter of *Plasmodium vivax* and Kitchen and Bradley (1936) showed that it was a possible carrier of *Plasmodium falciparum*. In addition to such laboratory evidence, Bang, Quinby and Simpson (1940) reported the capture of a specimen in nature harboring malaria plasmodia. In spite of these findings and the relative abundance of the species in some localities there has been very little work done on the biology of the species.

The studies reported here were made in Wabasha County, Minnesota, in the valley of the Mississippi River. Extensive slough areas and marshes make this locality especially favorable for anopheline breeding. Aquatic plants of both submergent and emergent types abound.

MATERIALS AND METHODS

Eggs of *walkeri* were obtained in the laboratory by confining the adults separately in shell vials (75 × 25 mm) or three or four in wide-mouth pint jars. The tops of the containers were covered with cheesecloth and a small amount of water maintained at the bottom. Adults were fed through the cheesecloth cover without difficulty. In the field, eggs were collected with a dipper and brought to the laboratory in jars. During the winter eggs were collected in the field where places of prolific breeding had been observed and well marked the previous season. Under conditions of this season, ice, snow and parts of emergent stems were taken into the laboratory and hatching awaited.

Larvae were collected with a dipper and transferred with a bent teaspoon to wide-mouth pint jars. Most of the rearing was done in the pint jars although individuals were reared in Syracuse watch glasses. Pulverized Ralston dog biscuit was used for food of the larvae with success.

Adult mosquitoes were collected by three methods. A light trap as designed by the workers in New Jersey (Mulhern, 1934) was run nightly. Adults when biting in the field were taken and excellent results were experienced by collecting those found resting on the sides of a barn and sheds at night where they had been attracted by the domestic animals, especially the pigs. A wide-mouth pint jar, modified for an aspirator to permit of larger catches before a transfer was necessary,

Received for publication, June 19, 1942.

* Contribution from the Department of Entomology and Economic Zoology, University of Minnesota. The writer wishes to express his sincere gratitude to Dr. William A. Riley for numerous suggestions on technique and procedure and his kind criticisms during the course of this investigation.

was used and a flashlight was found indispensable. On one favorable night over 400 specimens were taken in 15 minutes.

DESCRIPTION OF STAGES

Anopheles walkeri lays two kinds of eggs, the summer type which hatches without delay during the summer breeding season and the winter type which does not hatch immediately and is capable of withstanding winter conditions. The presence of these two forms was first noted and recorded by Matheson and Hurlbut (1937) and later fully described by Hurlbut (1938b). These two forms are shown in Fig. 1,

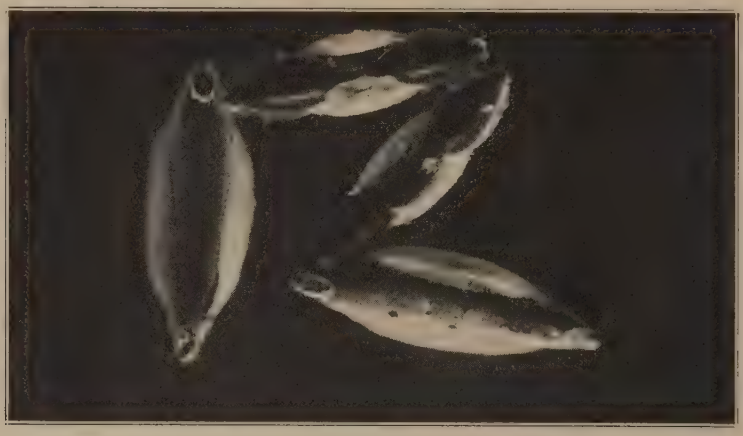

FIG. 1. Summer and winter eggs (the larger) of *A. walkeri.*

the winter eggs being longer, having longer floats and a reticulated exochorion over the dorsal surface. Measurements showed the summer eggs to be 664 $\mu \pm$ S.E. 0.03 in length and the winter eggs to be 767 $\mu \pm$ S.E. 5.4. These compare favorably with the measurements recorded by Hurlbut. Between the end of the period for the laying of summer eggs and the beginning of winter egg laying, transition forms may be laid which are intermediate between the summer and winter types both in size and structure.

The larvae are very similar to those of *A. quadrimaculatus* Say but may be separated from this species by the small distance between the basal tubercles of the inner anterior clypeal hairs. These hairs are sparsely branched toward the tip. Most of the specimens studied in this region conform to the so-called southern race as described by Bradley (1936). Hurlbut (1938a) has described the larval chaetotaxy of *walkeri* in detail.

The four larval instars are quite easily distinguished even in the field. This may not be done by observing body length alone as this dimension shows a gradual increase through the four instars from 0.9 mm to 7.7 mm. Measurements taken of head capsules of laboratory and field collected specimens show very definite size limitations for the various instars. The scatter diagram (Fig. 2) shows the measurement of the larval head capsules of specimens taken at random from field-collected specimens. The increase in size follows the well known Dyar's Law originally

applied to the head capsules of the LEPIDOPTERA. The average ratio of the measurements of successive instars is 1.6. The size of the head capsule, therefore, is sufficient to determine the stage of growth and application of this principle in the field soon enables the collector to identify readily with the naked eye the larvae to their proper instar.

A characteristic of the pupa which serves to distinguish this species from the

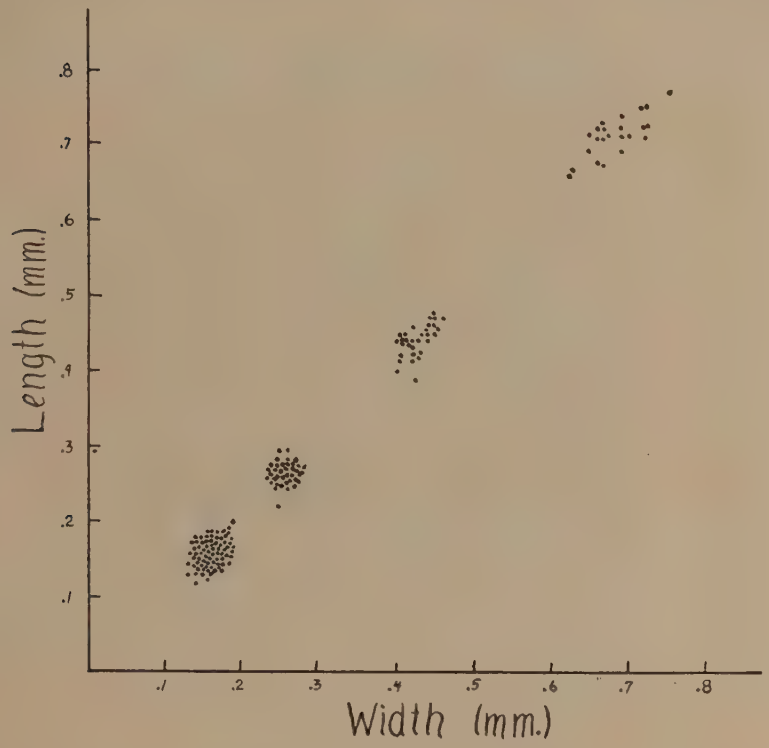

FIG. 2. Measurement of larval head capsules.

other anophelines in this area is the possession of a distinct serration along the outer margin of the paddle. This is shown in Fig. 3.

The adult female was first described by Theobald in 1901 and the male by Matheson and Shannon in 1923. In addition to the usual characters given in keys for *walkeri,* the specimens taken in this region are easily distinguished by their straw colored halteres, the other species possessing black halteres.

LIFE CYCLE

The chief difference in the known life cycle of *Anopheles walkeri* and that of other North American species of the genus is the ability to overwinter in the egg stage. Other phases of the life cycle are similar to those of other anophelines.

Number of generations: In these studies the light trap records for the years 1940

and 1941 were used, supplemented by records of the larval instars taken in the field during the season of 1941. The light trap was operated the entire night each night during the season (April 23 to September 30 in 1940 and May 2 to October 31 in 1941). Larvae were identified to proper instar at each collection in the field.

In 1941 only unhatched winter eggs were taken until April 9 when a few first instar larvae were obtained. Collections made subsequently showed the progress of the growth of these larvae. The adults first appeared in the light trap on May 20, 43 days after the first collection of larvae. A second flight of adults appeared the latter part of June, a third from July 20 through the first week in August and the fourth scattered from August 20 until the end of the season in October. The larval

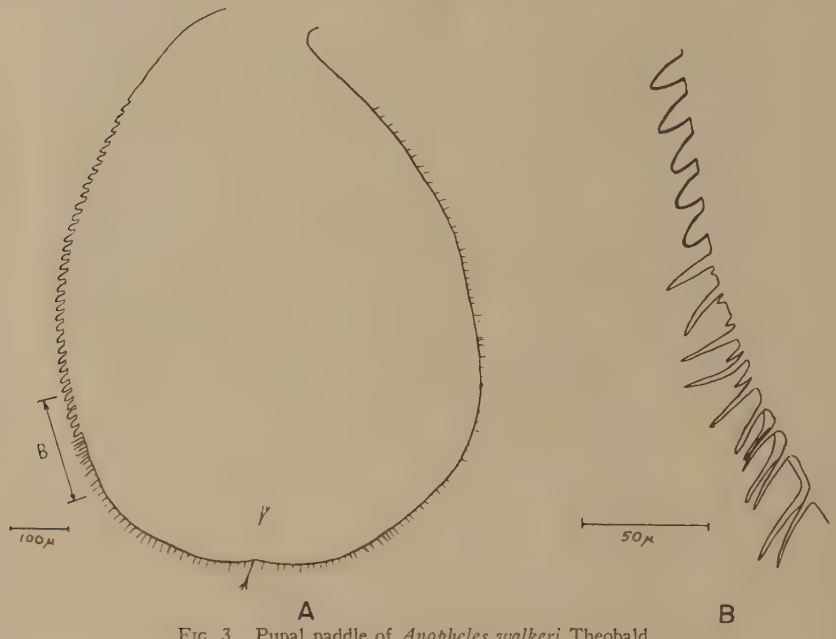

FIG. 3. Pupal paddle of *Anopheles walkeri* Theobald.

collections in each instance showed increased percentage of fourth instars just prior to the flight of adults appearing in the light trap and increase in the first instars immediately after the flights. Thus in the region of southeastern Minnesota there are four generations of *walkeri* per season.

Emergence: A tent of mosquito netting was placed over a square meter of water surface in the breeding area to test the rate of emergence. Over a period of 30 days a total of 115 *walkeri* (32 males and 83 females) were taken in the trap. This represents a per diem emergence rate of 2.8 mosquitoes per square meter of water surface. Collections were made twice daily, at dawn and again just before dusk for one week of the period to test differences in day and night emergence. During that week 21 (5 males and 16 females) emerged during the night and 24 (2 males and 22 females) emerged during the day, indicating that there is little if any preference for day or night emergence.

ECOLOGY

Temperature: Rate of development of *A. walkeri,* like other mosquitoes, varies greatly with the temperature. In the natural habitat there is considerable diurnal variation in temperature. In the laboratory, development from winter egg to adult required 58 days at 15° C, 42 days at 20° C, 31 days at 25° C and 21 days at 30° C. At 35° C the eggs hatched readily but the larvae died before reaching the second instar.

FIG. 4. Favored breeding place of *Anopheles walkeri* in *Leersia* and *Sagittaria.*

Winter eggs were kept in the laboratory at a temperature of 5° C and were kept moist. Experiments subjecting these winter eggs to low temperatures disclosed that they remained viable even when moist and kept at −21° C for a period of 72 hours. The larvae hatched and were perfectly healthy. Experiments at lower temperatures (−25° C and −27.2° C) showed that the larvae were injured. Although they were able to start hatching, they lacked sufficient strength to free themselves from the egg and usually died with the head or head and thorax outside the egg shell.

When the natural habitat of the winter eggs is taken into consideration, it is hardly likely that the temperature would fall low enough to injure the snow covered eggs.

Light: The larvae of *walkeri* are found among emergent vegetation but are more abundant where there is a plentiful supply of light than in densely shaded areas. It

is well known that the adults, although night fliers, very commonly bite in bright sunlight and that they are attracted to lights in large numbers.

Plants: The common emergent plants of this region furnish the habitat for *walkeri*. Among these, especially in the shallow portions of the sloughs, is the cut-grass *Leersia oryzoides*. The occurrence of *walkeri* larvae and *Leersia* together was so frequent that this grass proved to be a valuable indicator in making collections. Fig. 4 shows a typical breeding place in a dense growth of *Leersia* and *Sagittaria*.

Water level: A. walkeri normally breeds in water less than a foot in depth. During the 1940 season there was little fluctuation of the river level during the breeding season and there were numerous localities in the sloughs where *walkeri* could be taken at any time during the season. The water level during the last of June and the month of July was essentially the same in 1940 and 1941. At this time some larvae were taken. After the first of August the level dropped so that by the end of the month it had been lowered a foot. After collecting very few larvae on August 2, none of the previously favored places produced larvae. The water gradually receded from the breeding areas and became heavily overgrown with floating plants, principally *Spirodela*, thus precluding anopheline breeding. During this same season the breeding of *walkeri* continued unabated in a second area not influenced by the fluctuating level of the river channel. When a single lowering of the water level during a breeding season has such a deleterious effect, certainly a planned control based on this factor should prove very efficient.

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NUMBER OF LARVAE AND TIME REQUIRED TO PRODUCE ACTIVE IMMUNITY IN RATS AGAINST *TRICHINELLA SPIRALIS**

JACOB H. FISCHTHAL

Immunity acquired against *Trichinella spiralis* through recovery from a previous infection has been demonstrated in the rat by Ducas (1921), McCoy (1931), Bachman and Oliver-Gonzalez (1936), and Fischthal (1938), in the monkey by McCoy (1932a), in the pig by Bachman and Rodriguez-Molina (1933), in the guinea pig by Roth (1939), and in the mouse by Culbertson (1942). However, the question whether a single small dose of *T. spiralis* will confer immunity and the period of time necessary for its development have never been adequately considered. A preliminary description of this work has appeared in an abstract by Fischthal (1942).

METHODS

Four groups of 8 rats each were fed respectively a single dose of 80, 160, 320, and 640 larvae. Half the animals (4) at each dose level were given test doses of 10,000 larvae at 7 days (A), and the remaining half at 14 days (B). The plan was to examine in turn two of each group 7 days after their test dose for adults in the intestine (X), and the remaining two of each group 42 days after their test dose for larvae in the muscles (Z). In this laboratory the test dose herein used was found to be almost invariably fatal to non-immunized rats.

Controls in regard to the number of adults developing in the intestine were maintained by feeding two rats 80 larvae each, two 160 each, two 320 each, four 640 each, and two 10,000 each. Controls in regard to the number of larval worms in the muscles were not maintained since sufficient control data was accumulated in this laboratory over a period of years by Taylor (1932), Gursch (1933), Edney (1934), and Nolf and Edney (1937).

A total count was made to determine the exact number of larvae in the initial feeding of the rats, and the number of adults recovered from their intestines. Dilution counts (the average number of larvae in 10 one-cc samples multiplied by the total number of cc) were made to determine the test dose of 10,000 larvae, and the number of larvae present in the muscles of the rats.

RESULTS

Results with the experimental rats are shown in Table 1, where A–80–X indicates the rats given an initial dose of 80 larvae tested by 10,000 at 7 days, and examined for adults; B–80–X indicates rats of similar initial dose tested by 10,000 at 14 days, and examined for adults; A–80–Z indicates rats of similar initial dose tested by 10,000 larvae at 7 days, and examined for larvae in the muscles; B–80–Z indicates rats of similar initial dose tested by 10,000 larvae at 14 days, and examined for larvae in their muscles; etc.

Received for publication, June 22, 1942.

* This paper is based on a portion of the author's dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science at the State University of Iowa, June, 1938. Sincerest thanks are due Dr. L. O. Nolf under whose guidance this research was accomplished.

Results with the control rats given an initial dose and killed 7 days later for developing adults in their intestine are shown in Table 2.

TABLE 1

Key no.	No. days after test dose when examined	Adults recovered	Larvae recovered from muscles
A–80–X	3*	5,527	
	4*	5,473	
B–80–X	7†	3	
	7†	1	
A–80–Z	3*	3,185	
	3*	4,244	
B–80–Z	7‡	546	
	42†		133,550
A–160–X	4*	4,902	
	3*	2,592	
B–160–X	7†	4	
	7†	221	
A–160–Z	42†		
	3*	5,427	84,450
B–160–Z	20*		81,087
	42†		77,000
A–320–X	3*	5,749	
	4*	5,293	
B–320–X	7†	2	
	7†	33	
A–320–Z	6*	6,786	
	42†		86,200
B–320–Z	42†		103,300
	42†		113,700
A–640–X	4*	6,300	
	6*	4,425	
B–640–X	7†	5	
	7†	28	
A–640–Z	8*	6,828	
	8*	6,680	
B–640–Z	42†		153,950
	42†		149,450

* Died. † Killed. ‡ Killed accidentally.

DISCUSSION

Only 2 of the 16 rats given either 80, 160, 320 or 640 larvae and a test dose of 10,000 larvae 7 days after the initial infection survived. The 14 that died had a large number of adult worms present in their intestines. Six of these died on the third day after reinfection and the adults recovered varied from 2,592 to 5,749. Four of the 14 rats died on the fourth day after reinfection; the adults recovered varied from 4,902 to 6,300. Two rats died on the sixth day following reinfection and 4,425 and 6,786 adults, respectively, were obtained. The remaining 2 of the

TABLE 2

No. larvae fed	No. adults recovered	% larvae developing
80	24	30
80	34	42
160	22	15
160	32	20
320	125	39
320	172	54
640	52	8
640	26	4
640	491	77
640	534	83
10,000	3,303	33
10,000	3,189	32

14 rats died on the eighth day after reinfection; 6,680 and 6,828 adults, respectively, were obtained from their intestines. These results indicate that immunity had not been established in a period of 7 days, since, as shown in Table 2, the control animals show that approximately 33 per cent of the 10,000 larvae fed were recovered as developing adults 7 days after this feeding. McCoy (1932b) fed 3 rats that were not previously infected 10,800, 9,100 and 11,500 larvae, respectively. Five days later he killed them, recovering from their intestines 5,220, 4,640 and 6,420 adults, respectively. Since in the present series of experiments the majority of rats reinfected with 10,000 larvae died on the third, fourth and sixth days following reinfection, a comparison between the numbers of adults recovered here and the numbers recovered by McCoy shows that the rats reinfected after 7 days did not possess any degree of immunity at all. It appears from the experimental data available that these rats died in the intestinal phase of the disease. Post mortem examination showed intestinal hemorrhage and thrombosis of the mesenteric veins in the majority of cases.

The 2 rats that survived for 42 days the test dose given 7 days after the initial infection had in some way been able to resist the effects of this test dose. The number of larvae recovered from the muscles of the one surviving A–160–Z rat is more than is expected from a dose of 160 larvae, indicating that part of the 10,000 dose produced larvae which entered the muscles. However, the number recovered is approximately one-tenth the amount normally recovered from rats that had survived a single infection of only 9,000 larvae. The number of larvae recovered from the one surviving A–320–Z rat is the amount expected from a rat given a single infection of 320 larvae, indicating that probably no part of the test dose produced muscle infection. The resistance shown by these 2 rats may have been due either to a natural resistance against trichina larvae, to the rapid building up of a partial immunity in a seven-day period which was capable of preventing most of the test dose from producing muscle infection, or to a combination of both.

On the other hand, 15 of the 16 rats given either 80, 160, 320 or 640 larvae and a test dose of 10,000 larvae 14 days after the initial feeding survived. Nine of these rats were killed 7 days after reinfection. The adult worms present in the intestine varied from one to 546 in number. Seven of the 9 rats had less than 33 adults. A definite immunity is herein demonstrated, since, as shown in Table 2, approximately 33 per cent of the 10,000 larvae fed would be recovered as developing adults from non-immunized rats.

The remaining 7 rats given the test dose 14 days after the initial infection showed that immunity had been produced by the previous infection and that the rats were nearly perfectly protected against muscle invasion. It is noteworthy that after immunization by infection the immunity is effective against the intestinal phase of the parasite. The number of larvae recovered from the muscles of the one B–80–Z rat is more than is expected from a single 80 dose, indicating that part of the 10,000 had produced muscle infection; the number recovered, however, is approximately one-seventh of the amount usually obtained from rats that had survived a single infection of only 9,000 larvae. The numbers of larvae recovered from both of the B–160–Z rats are slightly above the expected amount from a single 160 dose; however, they are approximately one-tenth of the number usually recovered from rats that had survived a single dose of only 9,000 larvae. These two animals show that

a higher degree of immunity had been developed with a single dose of 160 larvae than with an 80 dose, although immunity still does not appear to be complete.

The numbers of larvae recovered from the muscles of both B–320–Z and both B–640–Z rats are the expected ones from the feeding of rats with 320 and 640 larvae, respectively, indicating that probably no part of the 10,000 test dose produced muscle infection. Here, complete immunity apparently has been accomplished with only a small number of larvae in a period of 14 days.

SUMMARY

It appears evident from the low numbers of adult *Trichinella spiralis* recovered from the intestines and the low numbers of larvae recovered from the muscles of rats that a single small dose of larvae is capable of producing immunity and that a period of approximately 14 days is adequate to develop this immunity. The feeding of 80 larvae produces a fairly high degree of immunity in 14 days; the 160 dose produces, in the same length of time, a higher degree of immunity; while both the 320 and 640 doses of larvae apparently produce complete immunity in a period of 14 days. The immunity produced is effective against the intestinal phase of the parasite. Little or no immunity is produced within seven days after feeding 80 to 640 larvae.

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MAINTENANCE OF A TREMATODE, *ASPIDOGASTER CONCHICOLA*, OUTSIDE THE BODY OF ITS NATURAL HOST*

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Aspidogaster conchicola is a trematode which usually spends its entire existence within the viscera of fresh-water mussels (Williams, 1942). Faust (1922) has recorded the same parasite from snails and from the digestive tract of a turtle in China. This species, which undergoes direct development with neither alternation of generations nor alternation of hosts, has been considered along with other species of related genera as constituting a distinct subclass of trematodes, intermediate between the MONOGENEA and the DIGENEA. Faust and Tang (1936) have proposed the name ASPIDOGASTREA for this subclass. A fairly full account of the life history stages of *Aspidogaster conchicola* adds support to the argument for recognizing this as a distinct subclass (Williams, 1942). The occasional occurrence of *A. conchicola* in other than the normal host offers evidence that this species is not rigidly fixed in its host relationships. However, the regularity with which it completes an entire cycle in the fresh-water mussels makes it seem plausible that presence in the turtle and in molluscs other than UNIONIDAE is accidental. Appearance in the body of a vertebrate might be explainable through the agency of food chains, wherein infected mussels, eaten by a turtle, permit the worms to become temporarily established in the body of an unusual host. Such secondary attainment of an additional host will be recalled as the basis commonly advanced to explain the origin of alternation of hosts among the parasitic worms.

Besides the forms occurring characteristically in molluscan hosts, there are other species and genera of ASPIDOGASTREA showing normal transitions toward the regular inclusion of a vertebrate host into which the worms must pass before they can reach sexual maturity. Thus in the genus *Cotylaspis,* one North American species, *C. insignis,* lives externally on the body of UNIONIDAE, while another species, *C. cokeri,* is found in the intestine of fresh-water turtles and has been recorded by Simer (1929) from the intestine of a paddle-fish (*Polyodon*). In yet another genus, *Cotylogaster,* an American species attains maturity in the intestine of the fresh-water drum (*Aplodinotus grunniens*). Of the food habits of this fish, Forbes and Richardson (1908: 324) state that it "feeds especially on mollusks, the shells first being crushed by the powerful, paved, millstone-like pharyngeal jaws." This memorandum suggests that in this species the vertebrate host might be superimposed upon a life cycle primitively complete in the molluscan body. This explanation seems particularly plausible since free swimming larval stages are wanting in the ASPIDOGASTREA as a possible means of affecting active transfer from molluscan to vertebrate host.

The versatility expressed by members of the subclass ASPIDOGASTREA in their host relationships suggests the possibility that physiological adjustment between the parasite and its host is not so rigorously fixed as it is among many trematodes. It

Received for publication, June 22, 1942.
* A contribution from the Zoological Laboratory of the University of Illinois.

therefore seemed probable that members of this group might offer satisfactory material for culture and infection experiments. The availability of *Aspidogaster conchicola*, on which life history observations were being conducted in this laboratory, lead to the selection of this species for further study of host relationships.

Various species of UNIONIDAE, collected from three Illinois streams, were the hosts used in this study. The heaviest infections were found in the mussels from the Sangamon River a few miles northeast of Mahomet, Illinois, and from the Wabash River at West York, Illinois. Lighter infections were encountered in the mussels from the Salt Fork, near Homer Park, Illinois. No attempt has been made to analyze the host relationship in terms of percentages of infection. Kelly has published extensive studies on this topic (1899) with particular reference to the mussel fauna of the Illinois River. In the present series of observations there did not seem to be any species of mussel immune to *Aspidogaster* and the individuals show very wide range in number of parasites without any apparent correlation with size or the locality from which the hosts came. *Amblema costata*, *Pleurobema coccineum*, and *Quadrula pustulosa* were fairly commonly and heavily parasitized in the collections from the Salt Fork and the Sangamon rivers but occasionally uninfected individuals of these species were encountered, while some individuals of *Lampsilis ventricosa* were heavily enough infected to yield numbers of worms sufficient for experimental purposes. Specimens of *Obovaria olivaria* and *Leptodea fragilis* were the chief sources of the worms from the Wabash River. In all of the collections, infections were rarely massive. On a few occasions, 30 to 40 *Aspidogaster* were removed from the pericardial and renal cavities of a single host individual but only rarely were there more than 20 in one mussel.

Two types of experiment were devised, one to test the possibility of maintaining individuals of *Aspidogaster conchicola* outside the body of the host and another type to determine, in preliminary manner, the possibility of individuals of this species surviving artificial introduction into the body of a vertebrate host. Some of the experiments here reported represent single sets of data while others are representative of several trials. None of the experiments has been repeated often enough to provide sound basis for generalizations, but the instances here selected are recorded in the hope that others may find in *Aspidogaster* a suitable material for a detailed program on trematode physiology.

I. THE MAINTENANCE OF *Aspidogaster conchicola* IN VITRO

Several series of experiments were conducted using various media, chief of which were physiological salt solution, Ringer's solution, tissue culture media and mussel blood. Living worms removed from the pericardial and renal cavities of freshly opened mussels, were placed with the medium in 8-dram vials. Most of the vials, after being tightly stoppered, were kept in a refrigerator at temperatures varying from 2 to 9 degrees C, though some were allowed to remain at room temperatures. Vials and instruments were sterilized and reasonable precautions were taken to prevent contamination, but the technique involving repeated washings of the worms was not resorted to, since Gatenby (1937) advises against it in directions for preparing tissue cultures. At reduced temperatures, the trematodes in the various media remained alive for periods varying from about 29 days in a tissue culture medium to a maximum of 75 days in mussel blood. These results, details of which are

presented below, indicate that *Aspidogaster conchicola* may be maintained over relatively long periods of time outside the body of its host and suggest the suitability of this species for physiological studies.

Physiological salt solution.—On October 5, 1940, three adult *Aspidogaster* were placed in a tightly stoppered vial of 0.75% physiological salt solution. After 20 days in the refrigerator all were alive and at room temperatures became active. On November 12, only one of the worms remained alive. After 38 days outside the body of the host, at temperatures between 2 and 9 degrees C, this individual remained inactive when removed to room temperature for examination.

Mussel Ringer's solution.—Six individuals of *A. conchicola* in a stoppered vial of mussel Ringer's solution were alive and apparently in good condition for 21 days (October 7 to 28). At the end of 39 days, at reduced temperature, all but one of the worms were dead. The dead individuals were removed and the one remaining worm lived only one day longer, or a total of 40 days outside the body of its host.

Hedon-Fleig's solution.—Gatenby (1937: 18) gives a formula for this solution, which is recommended for culturing snail tissues. This and another similar solution, which had been found suitable for culturing snail tissues, were tried as media for maintaining *Aspidogaster* in vitro. The results were about the same as for other synthetic media. In different experiments, at low temperatures, the worms lived in these media for from 29 to 38 days and at room temperatures lived for a maximum of 20 days.

Mussel blood.—Preliminary experiments had indicated that *Aspidogaster* may be maintained for 32 days at temperature from 2 to 9 degrees C in a mixture of body fluid and water from the viscera and mantle of freshly opened mussels. This suggested the possibility that pure blood of a mussel might be a satisfactory medium for maintaining these worms in vitro.

To secure a supply of undiluted mussel blood, an emery wheel was used to cut a window in the shell immediately over the pericardial cavity of the mussel (Ellis, Merrick and Ellis, 1931: 510). Heart and pericardial cavity were readily observable through the window. By use of a fine glass pipette, blood was drawn from the pericardial cavity, with a minimum of contamination. Repeated samples of about 1½ cc were taken from the same specimen at intervals of about one week without apparent ill effect upon the donor which was kept in aerated, running water at temperatures ranging from 13 to 21 degrees C.

On November 13, 1940, about 3 cc of mussel blood was placed in a sterilized vial, to which two adult *Aspidogaster* were added. As with the former experiments, the vial was placed in a refrigerator. At intervals of about two weeks, about 3 cc of fresh mussel blood was added, without removal of the blood previously present. On January 28, both worms were still alive. Previous to that date they had become very active at room temperature but on that date they were sluggish and on January 31 both were dead. These two individuals were kept alive in mussel blood for at least 75 days.

Bacterial contamination was fortunately at a low level in the tubes of mussel blood but three species of protozoa, abundant in the cultures, were identified by Dr. R. R. Kudo as *Hexamita inflata*, *Trepomonas agilis* and *T. rotans*.

The foregoing results indicate that mussel blood is far more satisfactory as a medium for prolonged observation of *Aspidogaster conchicola* outside the body of its host than any of the synthetic media that have been tried.

II. Experiments with *Aspidogaster conchicola* in vertebrate hosts

As mentioned in the introduction to this paper, Faust (1922) has recorded the occurrence, in nature, of *Aspidogaster conchicola* in the digestive tract of a turtle, while some other species of the subclass Aspidogastrea have life histories which normally seem to involve a vertebrate host. These facts, together with the observations on the readiness with which *A. conchicola* seems to adapt itself to existence away from the body of its normal hosts, made it seem desirable to try introducing this species artificially into vertebrate hosts.

On March 8, 1941, four adult worms were injected into the cloaca of each of two small turtles (*Graptemys* sp.). One turtle was killed and autopsied on March 10, but no trace of the worms could be detected in either the cloaca or the intestine. The second individual, autopsied on March 15, likewise gave evidence that the worms had been unable to become established in the cloaca or intestine of the turtles.

By means of a glass tube inserted well down the throat, six mature worms of *Aspidogaster conchicola* were introduced into the stomach of a male turtle (*Pseudemys rubiventris*), on March 8, 1941. Fourteen days later (March 17) the turtle was killed and upon examination one *Aspidogaster* was found clinging by its ventral sucker to the wall of the turtle's stomach. Frequent examinations of turtles from the same locality had previously established the fact that in nature *Aspidogaster* does not occur normally in turtles of this region. This observation, together with the fact that the turtle was without food from the start of the experiment, makes it seem reasonably certain that the recovered worm was one of those introduced experimentally.

The ability to survive, for at least a time, in the digestive tract of an unusual host, adds further evidence to the flexibility of host relationship expressed by *Aspidogaster conchicola* and makes it seem probable that the instances of occurrence in a turtle reported by Faust and in a paddlefish reported by Sinitzin were the results of accidental introductions when infected mussels were eaten by a vertebrate.

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NOTES ON HIPPOBOSCIDAE. 16. HIPPOBOSCIDAE FROM SOUTHERN BRASIL. WITH THE DESCRIPTION OF A NEW SPECIES OF *LYNCHIA*

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Some time ago the Deutsches Entomologisches Institut sent me nearly a hundred Hippoboscidae collected by Mr. Fritz Plaumann in Southern Brasil, at Nova Teutonia, State of Sa. Catharina (27°11' S. 52°23' W.). More recently additional Brasilian specimens were received from the same collector and others were presented by Mr. John Lane. These collections are particularly valuable for the careful host records. One of the species appears to be undescribed.

1. *Lipoptena* (*Lipoptenella*) *mazamae* Rondani.—The species is discussed at length in my "Monograph of the Melophaginae" (1942, p. 126). Nova Teutonia, 41 dealated females off *Mazama rufa* Rafinesque and 7 dealated females off *Mazama simplicicornis* (Erxleben) (= *rufa* Illiger) (F. Plaumann Coll.). Unpublished neotropical records: Mexico: Apamingan, State of Mochoacan, 1,200 ft., off *Odocoilleus virginianus* (subsp.?) (R. Traub Coll.). Venezuela: San Fernando, off *Odocoilleus cerunicornis* (*gymnotis* Wiegmann) (Fiasson Coll.).

2. *Ornithomya chloris* (Walker).—Nova Teutonia, two females off *Buteo magnirostris magnirostris* (Bertoni), one female off *Rhamphastos dicolorus* Linnaeus, nine females off *Urubitinga urubitinga* (Temminck) (= *Urubitinga zonalenca* Max), one female off *Micrastur rufficollis* (Vieillot), and one female off *Pyroderus scutatus* (Shaw) (F. Plaumann Coll.). Three flies off *Urubitinga urubitinga* and one off *Pyroderus scutatus* carried a mallophagan fixed by the mandibles to the tip of the abdomen. Unpublished neotropical records: Ecuador: Abinagua, 1,100 m. off a thrush (? *Turdus*), two flies, each bearing a mallophagan (W. Clarke-Macintyre Coll.). Chile: Valparaiso (Dr. Reed Coll.): Puerto Montt (A. Twomey Coll.). Brasil: Cara Pintada, State of Parana, off *Micrastur rufficollis* (Vieillot): Humboldt, State of Sa. Catharina, off *Rhamphastos dicolorus* (W. Ehrhardt Coll.). Venezuela: Colonia Tovar, off *Scops* sp. (P. Anduze Coll.). Guatemala: Uaxactun, Peten, off *Rhamphastos s. sulfuratus* Lesson (J. Van Tyne Coll.). This is the common species thus far called *O. confluens* in American literature; but Say's species of that name was off a wading bird (See J. Bequaert, 1940, p. 327; 1941, p. 289).

3. *Microlynchia erytrurelli* J. Bequaert (1938, p. 346, Figs. 1–3).—Nova Teutonia, one female off *Odontophorus capueira* (Spix), one female off *Crypturellus obsoletus* (Temminck), and three males and six females off *Columba rufina sylvestris* Vieillot (F. Plaumann Coll.). These flies all agree with the type. The occurrence on wild doves is unexpected, but apparently not accidental, as it was taken on at least three of these birds at different dates.

4. *Pseudolynchia canariensis* (Macquart) (Syn.: *maura* Bigot).—Ypiranga, State of S. Paulo, off domestic pigeons (J. Lane Coll.). Unpublished neotropical

Received for publication, July 1, 1942.

records: Mexico: D.F. (G. Lasmann Coll.). British Guiana: Georgetown, off domestic pigeon (J. Rodway Coll.).

5. *Stilbometopa podopostyla* Speiser.—Nova Teutonia, two females off *Columba rufina sylvestris* Vieillot, one female off *Tinamus solitarius* (Vieillot), one female off *Crypturellus obsoletus* (Temminck), and one female off *Chamaeza brevicauda* (Vieillot) (F. Plaumann Coll.). Ipaniry, State of Goyaz, off turkey (perú), and Goyaz, off dove (J. Lane Coll.). Unpublished neotropical records: Mexico: San José del Cabo, Lower California, off *Zenaidura macroura* (Linnaeus) (G. Augustson Coll.); La Majada, Munic. Apatzingan, 1,200 ft., State of Michoacan, off a dove (H. Hoogstraal and R. Traub Coll.). Venezuela: Las Robles, Guaira, off a white-tailed dove (P. Anduze Coll.). Paraguay: Villarrica, off domestic fowl (F. Schade Coll.).

6. *Lynchia fusca* (Macquart).—Nova Teutonia, one female off *Micrastur ruficollis* (Vieillot) and two females off *Buteo magnirostris magniplumis* (Bertoni) (F. Plaumann Coll.). Unpublished neotropical records: Panama: Sa. Clara, Chiriqui, off *Bubo virginianus mayensis* Nelson (M. E. McLellan Coll.). Brasil: Guarapuada, Rio Jordão, State of Paraná, off *Speotyto cunicularia grallaria* (Temminck); Cara Pintada, State of Paraná, off *Otus c. choliba* (Vieillot); Vermelho, State of Paraná, off *Strix hylophila* Temminck; Joinville near Humboldt, State of Sa. Catharina, off *"Bubo crassirostris"* (erroneous name) (W. Ehrhardt Coll.); São Paulo, off *Tyto alba tuidara* J. E. Gary (G. A. Allen Coll.; these specimens were recorded by error in 1933, p. 77, as from San José, California).

7. *Lynchia angustifrons* (van der Wulp).—Nova Teutonia, two females and one male off *Micrastur ruficollis* (Vieillot) and one female off *Ictinia plumbea* (Gmelin) (F. Plaumann Coll.). Unpublished neotropical records: Mexico: without more definite locality off *Accipiter striatus velox* (Wilson) (G. H. Merriam Coll.); Misantla, off "turkey buzzard" (W. Engelmann Coll.); Motzoronga, State of Vera Cruz, off *Trogon* sp. (L. Bruner Coll.). British Honduras: El Cayo, off *Glaucidium brasilianum ridgwayi* Sharpe, *Ictinia plumbea* (Gmelin) and *Odontotriorchis palliatus* (Temminck) (J. Van Tyne Coll.). Guatemala: Uaxactun, Peten, off *Accipiter bicolor* (Vieillot) and *Ramphastos s. sulfuratus* Lesson (J. Van Tyne Coll.); Olas de Moca, Dept. Solola (G. P. Engelhardt Coll.). British Guiana: Kartabo (W. Beebe Coll.); Issororo, N.W. district, off *Accipiter bicolor* (Vieillot) (G. M. Vevens Coll.). Brasil: Joinville near Humboldt, State of Sa. Catharina, off *Elanus* sp. and *"Bubo crassirostris"* (erroneous name) (W. Ehrhardt Coll.).

8. *Lynchia plaumanni* n. sp.

Lynchia plaumanni n. sp.
(Fig. 1)

Female.—Head (Fig. 1B) seen in front about one and one-third times as wide as high; frons at its narrowest about one and two-thirds times as wide as eye, measured along inner orbits scarcely longer than its greatest width at vertex, with sides markedly convergent toward lower third; inner orbits (parafrontalia) moderately wide, about one-third of width of mediovertex (frontalia) at its narrowest; frontal bristles few: a lower group of four (three very long) on gena and median row of three to five (one very long) near edge of mediovertex; one very long vertical bristle; postvertex (vertical triangle) much wider than long, the nearly straight or slightly wavy anterior margin longer than the sides, with one or more rudimentary ocelli or depressions taking their place, as described below (Figs. 1D–E); fronto-clypeus broadly and

rather shallowly emarginate medially, the antero-lateral angles moderately produced. Palpi moderately long, nearly half height of head.

Thorax (Fig. 1C) of usual shape; anterior margin nearly straight in the middle; humeral lobes slightly longer than wide, moderately prominent, bluntly rounded at apex, with one very long bristle and many shorter setae. Mesonotum on each side with two transverse patches of short setae, behind humeral lobe and before scutellum; a longer seta in prescutellar patch; a very long preälar and a similar postalar bristle on each side; dorsal portion of mesopleura (notopleura) with scattered short setae and one very long bristle near posterior corner. Scutellum semi-elliptical, hind margin evenly convex, fringed at edge with soft hairs and before this with a row of fine short bristles; one long scutellar bristle in each corner; metepimeron with a few, short setae. Legs without distinctive features; fourth tarsal segment of fore legs normal. Wing (Fig. 1A) rather short and broad; microtrichia covering the membrane except for small basal area in anal cell (Cu+1st An) and most of axillary cell (2d An); costa moderately swollen beyond tip of first longitudinal vein; first basal cell (R) very long, narrow, parallel-sided; second basal cell (M) slightly less than half length of first, closed by nearly vertical anterior basal cross-vein (M_3); subcosta (Sc) incomplete, not reaching costa; costa and basicosta densely setulose; all other veins bare.

Abdomen with usual broad, short, transversely losange-shaped basal, sclerotized tergite, bearing numerous short, stiff setae on sides and soft hairs in middle; preänal (dorsal) sclerite transverse, large, reniform, bearing few short setae and in each apical corner 7 or 8 very long, stiff bristles; remainder of dorsum forming one sclerotized area, without differentiated tergites, dull, microscopically cross-striate, almost without pilosity; sides and venter soft, uniformly and fairly densely covered with short setae; ventrally, two broad sclerites more or less defined on each side over basal half, hind margin of second evenly and broadly rounded off.

Male.—Extremely similar to female, even in width of frons and structure of abdomen; preänal sclerite has same shape; but basal sclerotized tergite is produced at sides into triangular lobes.

Length (of both sexes) from tip of fronto-clypeus to apex of scutellum, 2.7 mm; of wing, 5.5 mm; width of wing, 2 mm (total length of dry specimen, slightly over 4 mm).

Locality.—Brasil: Nova Teutonia, female holotype and male allotype off *Odontophorus capueira* (Spix); paratypes of both sexes off same host (2 ♀, 1 ♂), off *Crypturellus obsoletus* (Temminck) (1 ♀), and off *Chamaeza brevicauda* (Vieillot) (1 ♀); all collected by F. Plaumann. British Guiana: River Paruni, female paratype off *Crax nigra* Linnaeus (= *alector* Linnaeus). Bolivia: St. Elena, Huachi, Rio Beni, male paratype (W. M. Mann Coll.).

Type specimens.—Holotype and allotype at Mus. Comp. Zoöl., Cambridge, Mass.; paratypes at Deutsches Entom. Institut, Berlin-Dahlem, at British Museum, and at U. S. Nat. Mus.

An unusual feature of *L. plaumanni* is the presence of either rudimentary ocelli or ocellar pits, which could be traced in all 9 specimens (Figs. 1B and D–E). Their development varies. There may be only three pits in which no true ocelli can be seen, the pits being often more or less connected or sometimes forming one horse-shoe-shaped depression. The anterior ocellus can rarely be detected, but the two posterior ocelli are often outlined, though small and flat. It is extremely doubtful that any of them are at all functional. As *L. plaumanni* is in every other respect a true *Lynchia,* I can attach no particular taxonomic importance to these rudimentary ocelli. Nevertheless, *L. plaumanni* bridges the gap between *Lynchia* and the genus *Ornithophila* Rondani, at any rate as represented by *Ornithophila maquilingensis* Ferris (1924, p. 392, Figs. 1–2), of the Indo-Australian Region. Of that species I have studied specimens from the Philippines, Indo-China, Ceylon and Queensland, and it has also been reported from Java. It has the ocelli completely formed, though small, the anterior ocellus in particular being minute. Moreover, *L. plaumanni* is certainly distinct specifically, as it differs also in the extent of the bare area of the wing membrane. In *O. maquilingensis,* the wing is uniformly covered with microtrichia, except for a very narrow marginal area in the axillary cell. The paratype of *L. plaumanni,* from Bolivia, was mentioned by Aldrich (1923, p. 78) as running out in his key to *Ornithophila.*

In *Microlynchia*, as well as in some species of *Ornithomyia* (*O. fur* Schiner = *O. inocellata* Ferris), the ocelli are also in the process of disappearing. *L. plaumanni* and *O. maquilingensis* have merely retained one of the primitive characters of the ancestral stock of the genus *Lynchia*. Unless the unrecognized genotype, *Ornithophila vagans* Rondani, presents some other unusual feature, I should regard *Ornithophila* as no more than a subgenus of *Lynchia*. Unfortunately *Ornithophila* is the older of the two names and will have to replace *Lynchia*.

In the revised key to the New World genera of HIPPOBOSCIDAE, which I published in 1940 (pp. 312–313), the second alternative of couplet 12 should be amended to read "Ocelli absent or vestigial"; instead of "Ocelli absent."

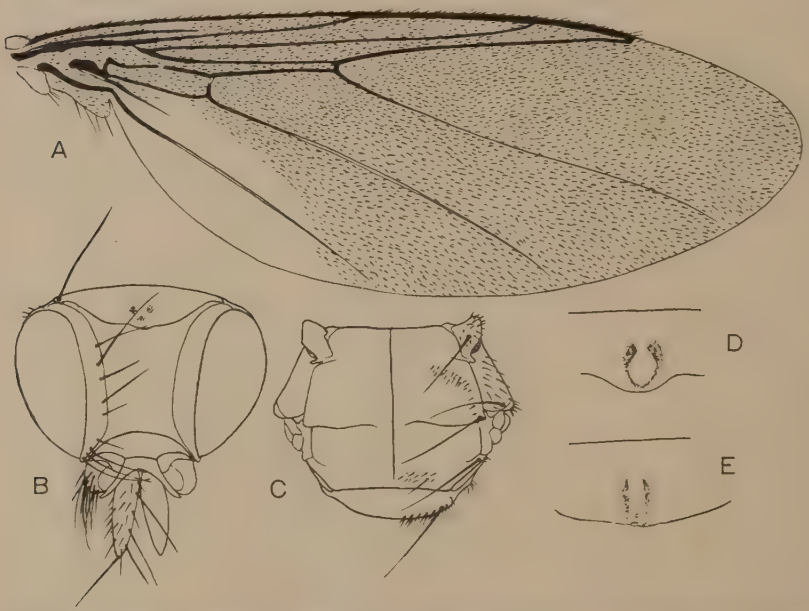

FIG. 1. *Lynchia plaumanni* J. Bequaert. A–C, female holotype: A, wing; B, head in front view; C, thorax from above. D–E, ocellar area of two paratypes.

The following key compares the described and recognizable small American species of *Lynchia* (wing 6.5 mm or less in length).

1. Frons very narrow in both sexes, at most as wide as an eye, usually narrower; inner orbits with few frontal bristles, placed in one row. Entire axillary cell and posterior half of anal cell bare. Palpi long, about one-half of the height of the head ..... *L. angustifrons* (v. d. Wulp).
   Frons always much wider than an eye in both sexes 2
2. Wing membrane covered with microtrichia except for a very narrow posterior marginal area of the axillary cell. Inner orbits with many frontal bristles in more than one row. Head subcircular. Palpi very short. Frons nearly three times as wide as an eye ..... *L. holoptera* (Lutz, Neiva and Costa Lima).
   At least posterior half of axillary cell devoid of microtrichia. Head distinctly wider than high 3

3. Anterior sclerite of mesonotum (prescutum, before transverse suture) with three strong bristles on each side, in the posthumeral patch of setae. Inner orbits with many frontal bristles in more than one row. Only entire axillary cell devoid of microtrichia. ..... *L. hirsuta* Ferris.

No strong bristles in the posthumeral patch of setae on prescutum 4

4. Only axillary cell either entirely or mostly devoid of microtrichia. Inner orbits with many frontal bristles in more than one row. Palpi short, much less than half the height of the head. No rudimentary ocelli. ..... *L. albipennis* (Say).

Most of axillary cell and part of basal area of anal cell devoid of microtrichia. Inner orbits with few frontal bristles in one row. Palpi long, nearly one-half the height of the head. Rudimentary ocelli present ..... *L. plaumanni.*

I regard *Olfersia botaurinorum* Swenk (1916), *Olfersia scutellaris* Swenk (1916) and *Olfersia palustris* Lutz, Neiva and Costa Lima (1915) as synonyms of *Lynchia albipennis.*

Olfersia propinqua Walker (1849), from Jamaica, was probably based on a small species of *Lynchia* (length of body 1½ English line = 3.17 mm) or perhaps on a *Microlynchia.* The description mentions no character now used to separate species in HIPPOBOSCIDAE.

9. *Ornithoctona erythrocephala* (Leach).—Nova Teutonia, one female off *Ictinia plumbea* (Gmelin) (F. Plaumann Coll.). São Paulo (J. Lane Coll.). Unpublished neotropical records: MEXICO: without more definite locality. PANAMA: El Volcan, Chiriqui, off a pigeon (C. B. Worth Coll.). VENEZUELA: Caracas (G. Umer Coll.); Merida, off a hawk and off *Accipiter chionogaster venezuelensis* Swann (P. Anduze Coll.); Naiguata Peak, off *Columba albilinea* Bonaparte (E. G. Holt Coll.); Zumbador, Estado Merida, off a pigeon (J. D. Smith Coll.); Rio Charaito, Estado Merida (G. Vivas-Berthier Coll.). BRASIL: Rio Grande do Sul (Stiegmayr Coll.). BOLIVIA: Incachaca, 2,500 m, off *Margarornis squamigera* (Lafresnaye and d'Orbigny) (J. Steinbach Coll.). PERU: Yurimaguas, E. Peru, off *Claravis godefrida* Temminck (= *Peristera geoffroyi* Temminck). ECUADOR: Baños, Oriente, off a small owl (W. Clarke-Macintyre Coll.).

10. *Pseudornithomyia ambigua* Lutz, Neiva and Costa Lima.—São Paulo, off a swallow (J. Lane Coll.). Unpublished neotropical records: VENEZUELA: El Valle, D.F., off *Iridoprocne albiventer* (Boddaert) (C. H. Ballou Coll.); Macuto, Caracas, off *Pygochelidon cyanoleuca* (Vieillot) (N. Dearborn Coll.).

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DEVELOPMENT OF EYE FLUKES OF FISHES IN THE LENSES OF FROGS, TURTLES, BIRDS, AND MAMMALS[1]

M. S. Ferguson[2]

About a century after von Nordmann (1832) first found strigeid metacercariae occurring in the eyes of fishes, La Rue, Butler, and Berkhout (1926) demonstrated that they were common habitats for species of these larval trematodes. Strigeid metacercariae have also been reported from the eyes of certain amphibians (Kelley, 1934). Various parts of the eye may be parasitized, depending upon the species of worm. This paper deals with metacercariae of the genus *Diplostomulum* that live only in the cortex of the lens, and is a report of experiments in which it has been found that worms ordinarily developing in the eyes of fishes, and in many cases causing complete blindness, will grow in the lenses of both cold- and warm-blooded animals representing four other classes of vertebrates.

The experiments were performed as an outgrowth of studies on the life history and control of eye flukes in the New Jersey State Fish Hatchery at Hackettstown. The trematodes represented at the Hatchery appear to resemble both *Diplostomum flexicaudum* and *D. indistinctum* (Ferguson and Hayford, 1941), and studies now in progress may show that these recognized forms are variants of a single species. All cercariae used in exposure experiments resembled closely, as far as morphology and behavior were concerned, the cercariae of *D. flexicaudum* (Cort and Brooks, 1928).

MATERIAL AND METHODS

The forked-tail strigeid cercariae were obtained from two sources; a small group of naturally infected snails (*Lymnaea stagnalis*) collected near Minneapolis, Minnesota, and laboratory-reared snails (*Lymnaea palustris*) experimentally exposed to miracidia of the Hackettstown trematodes. The snails were placed in milk bottles containing small amounts of aquarium water and cercariae which emerged were concentrated by centrifuging for a few minutes at low speed. The centrifuge tubes employed were made in this laboratory and are similar to a Hopkins vaccine tube (A. H. Thomas No. 3004) in that they are provided at the bottom with a short slender extension. The tubes have a strong constriction just above the extension and a greater capacity than the Hopkins vaccine tube. After centrifuging, the water in the large part of the tube can be poured out quickly without loss of the worms which collect in the extension.

The cercariae are plankton organisms which remain suspended in the water and the only swimming motion exhibited is a periodic and energetic movement upward. From the higher position they slowly settle after becoming quiescent. Natural fish hosts of *D. flexicaudum*, e.g., suckers, trout, minnows, etc., become infected as they swim into direct contact with the larval worms.

Bluegill sunfish acquire few eye flukes in nature and only a small number of

Received for publication, July 11, 1942.

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[2] The writer is indebted to Dr. F. G. Wallace, University of Minnesota, for supplying the naturally infected specimens of *Lymnaea stagnalis*.

cercariae reach the eye when these fish are exposed over several hours to thousands of cercariae in a small container. However, heavy infections can be obtained in the bluegill in at least three ways. (1) If hundreds of cercariae in a small amount of water are injected intraperitoneally into a fish, a varying number of worms usually reach the lens of each eye, where they develop normally and as rapidly as in the eyes of the more susceptible hosts. (2) Even greater numbers of cercariae will arrive in the lenses if a few drops of the suspension are injected into the orbit through the membrane surrounding the eyes. (3) When several drops of the concentrated suspension of cercariae are dropped down the open end of a pipette held tightly over the cornea for several minutes, many worms are also able to reach the lens. With the last method the pipette must be large enough to allow a considerable portion of the outer surface of the eye to be exposed to the cercariae. The pipette is placed in position and the cercariae are added to it while the fish is held out of water. The fish may then be held under water for 10–15 minutes with the pipette in place, by which time many cercariae will have had time to enter the eye.

This method of direct exposure of the eye to the larval worms was useful not only with the bluegill but with most of the animals mentioned in this paper. The diameter of the pipette employed was adapted to the size of the eye. Mice and rats were kept under anesthetic (ether) during the time of exposure, which for all the animals varied from 8 to 15 minutes. In the experiments only one eye was exposed.

Frog tadpoles used in the experiments were allowed to swim in water with the cercariae. Here, of course, both eyes could become infected. Other tadpoles which were examined as controls had no strigeids in their lenses.

EXPERIMENTS AND OBSERVATIONS

Employing the methods described above the following species of animals were exposed to the two strains of cercariae: frog tadpoles—*Rana pipiens* (probably); frogs—*Rana pipiens* (adults); turtles—small green, species (?), and painted—*Chrysemys picta* (small); chicks (2–4 days old); ducklings (3 days old); laboratory white mice (6 weeks old, and adults); white rats (young adults); guinea pigs (adults); rabbits (young adults); a sheep (1 year old); and a pig (2–3 months old). While all the animals did not develop metacercariae, representatives of each species, except the sheep and pig, became positive for eye flukes. Tables 1 and 2 summarize the data and show the interval of time between exposure to cercariae and examination of the lenses, the appearance of the lens at the time of removal from the eye, the number and condition of the worms found, and the size of some of the live metacercariae. Animals included in Table 1 were exposed to cercariae from the naturally infected Minnesota snails, while those in Table 2 were exposed to the Hackettstown material. Table 3 is a summary of all experiments and shows the numbers of animals which were positive or negative for metacercariae.

An experiment was attempted in which hundreds of cercariae were injected intraperitoneally into a guinea pig and four mice. After one day no worms had reached the eyes of any animal. This may mean that the cercariae are unable to survive and migrate in the bodies of these warm-blooded vertebrates. In another experiment several hundred cercariae were placed inside the lower eyelid of a rabbit. The procedure was repeated with two mice. Examination of the lenses two days later revealed no parasites. The cercariae must have been prevented from

TABLE 1.—*Results of experiments in which cercariae of* Diplostomum flexicaudum *from naturally infected* Lymnaea stagnalis *were applied to eyes*

Exper. No.	Species of animal exposed to cercariae	Interval between exposure of eye to cercariae and examination of lens	Condition of lens at time of examination	No. of metacercariae found		Range in body size of some live metacercariae after fixation†	
				Alive and active	Dead	Length	Width
		days				*mm.*	*mm.*
9E	Tadpole	23	Opaque and flaccid (one lens)	1	4		
5B	Frog	19	Normal	0	6		
5C	"	57	"	10	0	0.25–0.35	0.13–0.17
7F	Turtle (green)	35	"	1	0	0.32	0.13
11	Turtle (painted)	15	"	4	0		
3M	Chick	37	"	2	4	0.23–0.25	0.08–0.09
7E	"	45	"	0	3		
8C	Duckling	16	"	1	0	0.29	0.13
3A	Mouse	26	"	5	0	0.45–0.50	0.16–0.20
3B	"	33	Opaque and very flaccid	11	0	0.45	0.17
3E	"	48	" " " "	0	2		
3 G.1	"	48	" " " "	4	1		
3 G.2	"	97	Normal	1	0		
3H	Rat	27	Opaque and very flaccid	16	0	0.20–0.40	0.10–0.17
3I	"	34	" " "	11	0		
7A	"	48	" but firm	10	0		
7B	"	55	Normal	1	0		
3J	Guinea pig	33	Opaque and very flaccid	40*	10		
10A	" "	42	Normal	0	5		
3K	" "	63	Opaque and very flaccid	0	15		
3L	Rabbit	34	Normal	1	10		

* These metacercariae injected into duodenum of a 2-day-old chick. One normal adult worm with eggs recovered from intestine after 7 days. Metacercariae from experiments 3B, 3 G.1, 3I, and 7A were also injected into chicks, but no adult worms recovered.
† 10 measurements made where possible.

entering the eye either because of mechanical obstacles or because they were killed by the eye secretions.

In two instances an adult worm was obtained when live metacercariae from the lenses of a frog and a guinea pig respectively were injected directly into the duodenum of chicks. This indicates normal metacercarial development in these "abnormal" eyes, and is of particular interest because the chick is not the natural host of the adult trematode and very few adult strigeids are obtained even when flukes from the eyes of fishes are fed. Since the live metacercariae recovered from the eyes of various hosts in these experiments were normal in appearance and behavior, it may be assumed that numbers of them were physiologically mature when

TABLE 2.—*Results of experiments in which strigeid cercariae from experimentally infected* Lymnaea palustris *were applied to eyes*

Exper. No.	Species of animal exposed to cercariae	Interval between exposure of eye to cercariae and examination of lens	Condition of lens at time of examination	No. of metacercariae found		Range in body size of some live metacercariae after fixation†	
				Alive and active	Dead	Length	Width
		days				*mm.*	*mm.*
6A	Tadpole	6	Normal (2 lenses)	2	0		
6B	"	18	" (2 ")	3	0		
2A	Frog	19	Opaque but firm	175	0	0.18–0.22	0.07–0.12
2B	"	50	" and very flaccid	40*	15	0.26–0.33	0.08–0.10
1D	Mouse	55	" but firm	4	0	0.30–0.37	0.10–0.17
3A	Rat	50	Normal	1	1	0.30	0.12
3B	"	57	"	5	0		

* 30 of these metacercariae injected into duodenum of a 2-day-old chick. One normal adult worm with eggs recovered after 7 days.
† 10 measurements made where possible.

TABLE 3.—*Summary of all experiments showing animals which were positive or negative for metacercariae*

Species of animal exposed to cercariae	Cercariae from *Lymnaea stagnalis*		Cercariae from *Lymnaea palustris*	
	Positive	Negative*	Positive	Negative*
Tadpole	1	3	2	3
Frog	2	0	2	0
Turtle (green)	1	0	0	1
Turtle (painted)	1	0	0	0
Chick	2	0	0	2
Duckling	1	1	0	0
Mouse	5	6	1	2
Rat	4	0	2	0
Guinea pig	3	3	0	0
Rabbit	1	2	0	2
Sheep	0	1	0	0
Pig	0	1	0	0

*In all cases the lenses were normal in appearance.

removed from the lenses several weeks after exposure of the eyes to cercariae.

The mortality which occurred among the metacercariae in some of the "abnormal" lenses after they had reached morphological maturity might have been greater if the worms had been allowed to remain in the lenses for longer periods, such as several months. Dead eye flukes were usually disintegrating when recovered from the lenses. Strigeid metacercariae do live for many months, perhaps years, in the eyes of fishes, particularly when they are few in number and when the lens is not severely damaged. However, dead and disintegrating metacercariae may be found in fish eyes when the lens has become flaccid and opaque. There is an indication (Table 1) that the eye worms survive longer in mice and rats than in guinea pigs.

Sizes of metacercariae recovered alive and fixed in hot alcohol-formalin-acetic solution were studied (Tables 1 and 2). There is a considerable variation in eye flukes from a single lens just as between metacercariae from the eyes of different species of animals. The range in size of the worms in Table 1 is slightly greater than of those in Table 2. This may have been due to the fact that cercariae released by *L. stagnalis* were larger than those obtained from infected *L. palustris*. Then too, there is still some question as to the specific identity of the cercariae from the two snail hosts which represent strigeid material from Minnesota and New Jersey. The evidence in Tables 1 and 2 suggests it would be premature to state that any marked size variations exist between the worms from the eyes of cold- and warm-blooded animals when they were exposed to cercariae from the same source. This is further emphasized by the fact that metacercariae from the eyes of rainbow trout, bluegill sunfish, and blackhead minnows exposed to cercariae from either *L. stagnalis* or *L. palustris* varied considerably among specimens in each collection. There was not, however, a marked variation in the size range of worms in the different collections from the various species of fish hosts. These sizes correspond rather closely with many of those given in Tables 1 and 2.

Undoubtedly, the environment in which the metacercariae develop and the source of the cercariae are important factors in determining the size attained by the mature eye flukes.

Pathological Effects of the Eye Flukes

The lenses were often seriously affected by the presence of the eye flukes unless the number present was small (Tables 1 and 2). In some cases where only a few metacercariae were present much harm was done. The initial damage to the lenses

brought about an opaqueness of the cortex and nucleus. In those lenses which became flaccid the cortical substance was partially liquefied. The lens capsule then served to retain this material and the more solid nucleus of the lens.

In some of the rabbits, guinea pigs, rats, and mice, the corneas became opaque within a day or so after exposure to the cercariae. This condition usually persisted. Some of these eyes contained damaged lenses, but in others the lenses were normal in appearance. Corneal damage may have occurred at the time of exposure due to irritation caused by the piperne and cercariae, or secondary organisms carried in by the worms as they penetrated the eye.

The affected eyes resembled closely those found in fishes parasitized by numbers of metacercariae as reported by Ferguson and Hayford (1941). However, diseased lenses usually became evident much earlier in the warm-blooded animals than in fish and in these warm-blooded forms severe damage to the lens resulted from the presence of only a few worms, whereas it usually required large numbers of metacercariae to cause blindness in fishes.

DISCUSSION

Information at hand appears to show that so far as fishes are concerned the lens of the eye is the only tissue in which the cercariae of *D. flexicaudum* can survive and develop. The larval trematodes will penetrate eyes of fishes from which the lenses have been removed, but do not survive more than about a day.

The fact that these metacercariae will develop in animals representing so many classes of vertebrates suggests that the lens materials in the various eyes are, to a certain extent, rather similar in nature. As a matter of fact, tests show that the lens proteins of more or less closely related vertebrates within a class are apparently similar (Markin and Kyes, 1939), but differences have been demonstrated in lens materials of animals belonging to separate classes (Ecker and Pillemer, 1940). These chemical differences are evidently not great enough to make the lens substance of any of the particular animals mentioned here unsuitable nourishment for the developing metacercariae.

Obviously the observations presented here indicate that the metacercaria of *D. flexicaudum* is not strictly host-specific. This is further emphasized in the papers of Cort and Brackett (1937) and Olivier (1940a), who reported that metacercariae of this form sometimes develop precociously in old sporocysts of lymnaeid snails and that these will develop into normal adult trematodes in the chicken's intestine. In certain snails, therefore, some physiological condition permits the larval worms to develop beyond the cercarial stage, and one is tempted to speculate that the environment in older sporocysts in the invertebrate host is similar to that in the cortex of the vertebrate lens.

It is not unique among strigeid trematodes that the metacercariae of *D. flexicaudum* can live and grow in the eyes of such a variety of hosts. Szidat (1929) reported metacercariae of *Cotylurus cornutus* from the hermaphroditic glands of snails and reproductive organs of leeches. A considerably wider range of intermediate hosts for a strigeid metacercaria is listed by Wallace (1939). In China, the tadpoles and adults of several species of frogs, snakes of several species, a skink, and a shrew were found to be naturally infected with larval *Pharyngostomum cordatum*. Metacercariae of this species recovered from frogs and snakes were fed

to rats, chicks, ducklings, and an owl. Those which survived did not develop into adults, but migrated into the muscles and again became encapsulated. The frog is perhaps the only required intermediate host, and snakes may acquire their infection by eating frogs which harbor encapsulated metacercariae already fully developed. Whether cercariae develop only in frog tissues remains to be seen, but it is evident that metacercariae can survive in the gut of many hosts, migrate out into the muscles, and there find locations suitable for survival even though some of the hosts are warm-blooded.

Representatives of various invertebrate and vertebrate classes have also been reported as hosts for the metacercariae of *Strigea vaginata*, *Apatemon gracilis*, and *Prohemistomum vivax* (Walton, 1938; Olivier, 1940b).

Since the eye flukes of fishes develop in the lenses of mammals, and frequently cause blindness, the question arises whether these worms are able to establish themselves and do damage in the eyes of man. There is probably ample opportunity for large numbers of bathers to be exposed to the cercariae of *D. flexicaudum* in the lakes of Michigan, Wisconsin, Minnesota, and surrounding regions both in the United States and Canada (Cort, McMullen and Brackett, 1937). Dr. A. C. Woods, Ophthalmologist-in-Chief, Johns Hopkins Hospital, in a personal communication, informed the writer that there appears to be no published record in this country of larval trematodes having been found in human eyes. Ward (1918) cites no cases for North America, but both he and Salzer (1907) mention a few instances in which trematodes have been found in the eyes of Europeans. None of the worms mentioned appears to be a strigeid. It is possible that the ocular secretions and thickness of the tissues of the eye in larger animals may be effective barriers to the occasional cercariae which might otherwise be able to parasitize people while bathing near the shore in freshwater lakes. It should be recalled also that there was no survival and migration to the eye when cercariae were injected into mice and one guinea pig, nor was there penetration of the eye by large numbers of worms placed under the lower eyelid of mice and a rabbit. That the cercariae of *D. flexicaudum* may be occasionally dangerous to man seems therefore to be rather remote.

If, however, eye flukes ever become established in the eyes of bathers their presence might not be detected for some time. Furthermore, aside from irritation that probably would result from the penetration of the cercariae through the tissues, no apparent damage to the lens might be caused by developing metacercariae (Table 1, experiments 3A, 10A, 3L). On the other hand, a severe disturbance in the lens might result from the presence of only a few worms (Table 1, experiments 3E, 3G.1). The experiments summarized in Table 1 show also that in warm-blooded animals metacercariae may die and begin to disintegrate in lenses that are either normal in appearance (experiment 3L) or severely damaged (experiment 3K). If one of the latter lenses were examined after the metacercariae had died and disintegrated, little trace of the agent causing the disease would remain. Salzer (1907) described the repair occurring in slightly damaged lenses of fish in which strigeid metacercariae had died and disintegrated. A similar study was not made with the diseased eyes in the warm-blooded animals employed in the experiments. That repair of the lens and its return to an apparently normal condition might occur in some of these warm-blooded forms, and even in man, are possibilities.

Until proved a non-existent danger, and in view of the earlier European records,

human parasitization with metacercariae of *D. flexicaudum* can not be considered impossible.

SUMMARY

Cercariae of the strigeid trematode, *Diplostomum flexicaudum,* and other cercariae considered to be probably the same species, were able to penetrate and grow into metacercariae in the lenses of tadpoles, frogs, turtles, chicks, ducklings, mice, rats, guinea pigs, and rabbits. These metacercariae were normal in appearance and behavior and were similar to those developing in the eyes of fishes, the natural intermediate hosts. Metacercariae from the lenses of a frog and a guinea pig were infective for baby chicks, and normal adult worms with eggs were recovered from the bird intestine after one week. Blindness was caused in many of the infected eyes. Certain experiments and an apparently complete lack of records in medical literature suggest that the possibility of these eye flukes causing disease in man is rather remote. However, it remains to be proved that such a danger does not exist.

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A NEW TREMATODE, *DICTYANGIUM CHELYDRAE* (MICROSCAPHIDIIDAE = ANGIODICTYIDAE), FROM THE SNAPPING TURTLE, *CHELYDRA SERPENTINA*

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Looss (1899) designated *Monostoma reticulare* van Beneden, 1859 and *M. proteus* Brandes, 1891 as types of the two genera *Microscapha* and *Baris,* respectively. Since both generic names were preoccupied, Looss (1900) proposed the names *Microscaphidium* and *Deuterobaris* to replace the homonyms and he included the two genera in the subfamily MICROSCAPHIDIINAE. Later, Looss (1902) designated the three species *Microscaphidium linguatula* (Looss, 1899), *Microscaphidium sagitta* (Looss, 1899) and *Microscaphidium parallelum* (Looss, 1901) as types of the new genera *Polyangium, Octangium* and *Angiodictyum.* In this paper he included *Microscaphidium, Angiodictyum* and *Polyangium* in the subfamily MICROSCAPHIDIINAE while *Octangium* and *Deuterobaris* were designated as types of the subfamilies OCTANGIINAE and DEUTEROBARIDINAE. The three subfamilies were included in a family which he named ANGIODICTYIDAE. Since *Angiodictyum* is not the type of the subfamily to which it belongs, it can not be the type of the family and the name ANGIODICTYIDAE is invalid. In an attempt to rectify the incongruity, Poche (1925) proposed the subfamily ANGIODICTYINAE, with the following explanation, "Den Namen *Angiodictyinae* führe ich an Stelle von Microscaphidiinae Looss (1900, p. 605; 1902, p. 699) ein, weil diese Unterfamilie die typische Gattung der Familie Angiodictyidae, nämlich *Angiodictyum* Looss, enthält und somit die typische Unterfamilie dieser Familie ist und es mit dem Begriff 'typisch' unvereinbar ist, dass die typische Gattung der typischen Unterfamilie einer Familie eine andere ist als die der Familie." Poche thereby renamed a previously validly named subfamily and ANGIODICTYINAE is a subjective synonym of MICROSCAPHIDIINAE. A discussion of the taxonomic principle involved here and an opinion by Professor C. W. Stiles on it appear in a paper by Stunkard and Nigrelli (1930). Travassos (1922) proposed the name MICROSCAPHIDIIDAE and has used it in his later papers. Although a history of the case shows that it is the only available one, most subsequent authors have accepted the name ANGIODICTYIDAE.

All of these trematodes studied by Looss were from the intestine of marine turtles. In his monographic report, Looss (1902) gave a detailed account of their morphology and recognized for the first time that the vascular channels which ramify through their bodies represent two distinct and independent systems. One set of tubules was identified as a portion of the excretory system, whereas the other was described as a "Lymphgefässystem."

Since the monograph by Looss, there have been few additions to knowledge of the group. Odhner (1911) reported a new but undescribed and unnamed species from *Teuthis* sp., a fish from the Red Sea, and asserted that the angiodictyids are amphistomes in which the acetabulum has been lost. The notch at the posterior end of forms such as *Octangium* was regarded as a result of the reduction and dis-

Received for publication, July 22, 1942.

appearance of a terminal sucker. He pointed out that the morphological agreement of *Distomum quadrangulatum* Daday, 1907 with the amphistomes and angiodictyids may be an indication of genetic relationship. Travassos (1922) created the new genus *Parabaris* to receive *P. parabaris,* a species from the intestine of a Brazilian fresh-water fish, *Piaractus brachypomus.* Referring to *D. quadrangulatum,* Travassos, Artigas and Pereira (1928) stated that this species may be congeneric or even specifically identical with *P. parabaris.* Subsequently Travassos (1934) definitely assigned *D. quadrangulatum* to *Parabaris,* and in accord with the suggestion of Odhner, he included the microscaphids, the amphistomes and other supposedly related groups in the PARAMPHISTOMOIDEA. Poche (1925) created a new genus *Nephrobius* for two specimens labelled, *"Monostoma nephrit. ren. Colymb. arctici"* which he found in the Graz collection. Goto and Ozaki (1929) described *Hexangium sigani* from the intestine of *Siganus fuscescens,* a Japanese marine teleost. The most recent contribution is by Price (1937) who created two new genera; *Octangioides* to contain a species from the Mexican fresh-water turtle *Dermatemys mawaii,* and *Hexangitrema* for a species from the black angelfish *Pomacanthus arcuatus.* In a footnote, Price suppressed *Nephrobius* as a synonym of *Polyangium.*

The examination of 79 specimens of *Chelydra serpentina* collected in Louisiana yielded 16 infections and a total of 149 specimens of a new microscaphid trematode for which the name *Dictyangium chelydrae* is proposed. The turtles were large, with shell lengths of 6 to 11 inches. The worms were found only in the initial portion of the large intestine. The number of mature specimens found in individual turtles was always small. The largest number, 36, was found in a turtle dissected in May, 1942. It contained 8 mature and 28 juvenile worms.

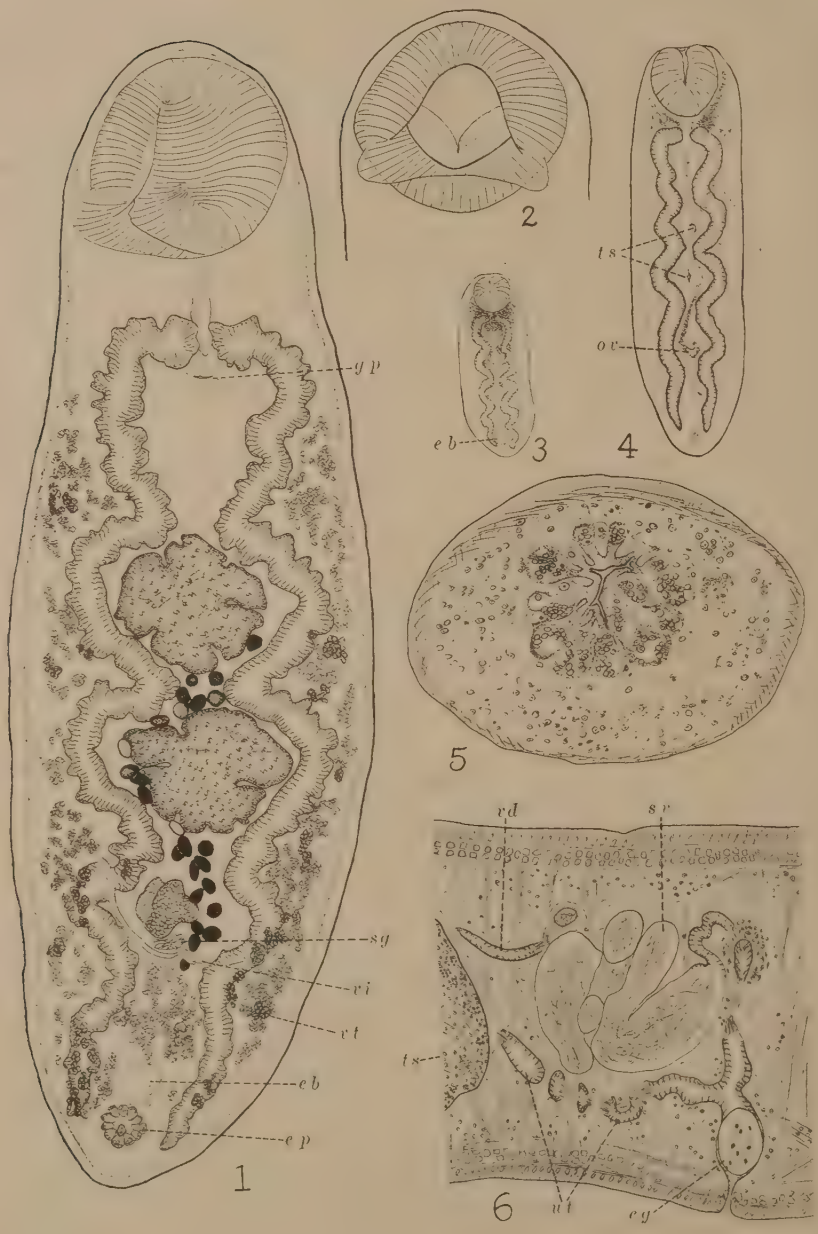
Dictyangium chelydrae n. g., n. sp.
(Figs. 1–6)

The worms are elongate, rounded at the ends, with almost parallel sides. They are flattened, convex dorsally and concave ventrally. On contraction, the anterior and posterior ends bend ventrally, forming an elongate, cupuliform depression. For this reason it is very difficult to secure well extended specimens and whole mounts were made only from worms fixed under the pressure of a cover glass. As they move forward, the worms turn spirally on the long axis. Sexually mature specimens may extend to a length of 6 mm and contract to a length of 2 mm. In width they measure from 0.5 to 1.5 mm. Gravid specimens, fixed, stained and mounted, measure from 2.5 to 4 mm in length and 0.6 to 2 mm in width. In young worms (Figs. 3, 4) the gonads are represented by small clusters of germinal cells, and the dorsal surface of the body is underlaid with diffuse pigment which is most abundant at the level of the intestinal bifurcation and extends posteriad in a V-shaped pattern. There are no cuticular spines, but the musculature of the body wall presents a cross-hatched appearance which suggests a spined cuticula. As in other members of the family, the musculature of the body wall is well developed and bundles of longitudinal fibers are present inside the diagonal layer. The dorsoventral muscles are numerous and very strong.

The anterior sucker is 0.45 to 0.6 mm in diameter and usually spherical in outline. Details of its structure are shown in Fig. 2. Paired, conical to auriculate, ventrolateral papillae may be protruded as lobes, but there are no retrodorsal diverticula and the wall of the sucker shows no indication of such evaginations. It is limited by strong external and internal membranes and the fibers are arranged in a definite pattern. Branched fibers extend from the internal to the external membrane and parallel meridional fibers extend around the internal and external faces. In addition, a band of fibers passes around the sucker, dividing it into cortical and central portions, with most of the nuclei situated in the cortical zone. The sucker is anchored to the body wall by protractor and retractor muscles, and small lip-like projections of the body wall may be protruded around the mouth. The oral opening is triangular, wider anteriorly and tapering more or less regularly to a point posteriorly. There is no sphincter and the mouth can not be completely closed. Instead, the sides converge from posterior to anterior, leaving a slit-like aperture with a

triangular opening in front. The absence of a sphincter also imparts a certain flaccidity to the sucker, which under pressure often collapses (Fig. 1). The lumen of the sucker is triangular and tapers retrodorsally to become continuous with that of the esophagus. The anterior end of the esophagus is spanned dorsally by the commissure of the nervous system and it then makes a U-shaped bend, passing ventrally and then dorsally where it communicates with the two lateral digestive ceca. The cuticular lining of the esophagus separates the epithelia of the ceca so that they are not continuous. The posterior end of the esophagus is frequently somewhat dilated, but there is no muscular esophageal bulb, and no appreciable increase in thickness of either the circular or longitudinal coats. The ceca have saccate walls and end blindly near the posterior end of the body. Their course is sinuous but characteristic; they bend laterally leaving wider median areas at the levels of the copulatory organs, each of the testes, the ovary, and the excretory bladder. This pattern is manifest in young specimens (Figs. 3, 4), even before there is any trace of the reproductive organs. Histologically, the longitudinal and circular muscles, reduced in size and number, extend from the esophagus along the walls of the ceca. The epithelium has a deeply staining basal zone in which nuclei are conspicuous but in which cell boundaries are indistinct and the cells bear long cytoplasmic projections, striated in appearance and resembling cilia, which extend into the lumen.

The excretory system is very complicated. It consists of a tubular collecting system which originates at the flame cells, a series of vesicular channels, and a pulsatile bladder. The pore is median, dorsal, near the posterior end of the body. The musculature of the body wall is modified to form a sphincter around the opening and a duct, lined with cuticula, extends ventrad and anteriad to open into the ventroposterior end of the excretory bladder. The internal end of the duct is provided with a weak sphincter. From the duct, near the external sphincter, a dozen or more small tubules radiate to open into flask-shaped enlargements (Fig. 5) that comprise the "rosette." The tubules are lined with cuticula and the enlargements are more or less completely filled with deeply staining nuclei. Cell outlines were not observed, but open spaces in the enlargements may contain traces of a coagulated material. The bladder has a very thin wall which is much folded when collapsed. It is lined with a low epithelium. In living specimens the bladder and vesicular channels contain a fluid in which fine droplets are suspended, and movement of the droplets facilitates observation and makes it possible to follow the course of the channels. On each side the bladder communicates with a thin-walled, irregularly-shaped sac. The connections were observed first in living specimens and confirmed later by the study of serial sections. From the ventral part of the sac, a main channel passes forward and another backward. The posterior one gives off a branch which passes posteriad and subdivides to form smaller branches, while the main trunk courses laterad, behind or below the cecum and passes forward along its lateral face. The anterior one gives off a branch which then subdivides, but the trunk turns dorsad to join the main dorsal longitudinal channel which extends forward but also continues backward to partially over-lie the thin-walled sac that opens into the bladder. Actual communication with the sac, although indicated, was not demonstrated. Observation of this region is difficult since the bladder is surrounded by vitelline follicles, the body is opaque, the bladder contracts with explosive suddenness, the bladder may be partially over-lapped by the lateral sacs and these in turn by the intestinal ceca, while the parenchyma is so loose that intercellular spaces may be confused with portions of the excretory system. The vesicular portion of the excretory system consists of the two main trunks on each side of the body, one median and dorsal, the other lateral to the cecum, together with a large number of branches from each of them. The branches may subdivide and usually they end blindly with expanded tips. When collapsed, they are hard to follow in sections. Although the position of the larger branches is relatively constant, there is much variation, especially in the smaller channels. Diverticula from the lateral and median trunks of one side may communicate in the anterior half of the worm and communications between the two sides may occur at the level between the testes, near the bifurcation of the digestive tract, and the channels of the two sides are always united by a loop which passes anterior and dorsal to the sucker. The vesicular channels are lined with a low epithelium and the walls appear to be membranous, although the lumen may expand and contract without any apparent movement of the surrounding tissues. This observation suggests that the vessels have muscular elements in their walls, although changes in their size and shape may be due to contraction of fibers in the adjacent parenchyma. Movement of fluid in the tubules is often rapid and the flow may be in either direction, although eventually the fluid passes backward and out of the excretory pore. The vesicle is well developed in young individuals (Fig. 3), but the channels are so small that it is difficult to trace them. Near the anterolateral margins of the digestive ceca, collecting ducts from the flame cells open into the vesicle. Each duct passes backward and mediad, accompanied by a recurrent tubule which joins it in front of the anterior testis. Each collecting duct also receives a branch at the level between the testes, another just posterior to the posterior testis, and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another behind the ovary. These secondary tubules give rise to a very large number of capillaries, each of which ends in a flame cell. The flame cells are small and very numerous; more than 30 were counted on one side between the sucker and the level of the anterior margin of the cephalic testis, but despite repeated attempts it has been impossible to determine the precise pattern of branching in the tubules.

A lymph system, which supposedly is characteristic for the family, is not present.

The testes are large lobed organs, the anterior one situated near the middle of the body and the posterior one a short distance behind it. They are approximately the same size and measure from 0.2 to 0.5 mm in diameter. Usually both are actively functional although vitelloid droplets were observed in the testes of 3 out of 15 worms sectioned and in 2 of 28 mature specimens mounted in toto the anterior testis is degenerate. The vas efferens from the posterior testis arises at its median, anterior, dorsal margin and passes forward and ventrad. It then loops dorsad and near the posterior margin of the anterior testis is joined by the vas efferens from that gonad. The vas from the anterior testis arises at the dorsal margin of the testis and passes posteriad, joining the one from the caudal testis to form the vas deferens which passes forward dorsal to and on the right side of the anterior testis. In front of the anterior testis this sperm duct opens into a large, thin-walled, much coiled seminal vesicle which occupies the region between the anterior testis and the level of the genital pore. The seminal vesicle (Fig. 6) is continued by a thick-walled duct which turns ventrad and opens into the genital atrium. A portion of this duct, about the second quarter of its length, is surrounded by secretory cells. There is no cirrus sac or cirrus.

The ovary is situated slightly to the right of the median line, approximately its diameter behind the posterior testis. It is lobed and measures from 0.1 to 0.2 mm in diameter. The oviduct arises at the dorsal margin, coils mediad and posteriad where Laurer's canal diverges and passes in a sinuous course to open at the dorsal surface of the body. After the origin of Laurer's canal, the oviduct turns ventrad, receives the common vitelline duct, and coils posteriad, dextrad and ventrad where it enters the shell gland and expands to form the ootype. The uterus emerges from the left side of the shell gland, coils backward and then forward on the left side of the ovary, the right side of the posterior testis, the left side of the anterior testis, and then below the seminal vesicle to the genital atrium where it enters behind the opening of the male duct. The atrium (Fig. 6) is shallow and the genital pore is median, ventral, immediately behind the bifurcation of the digestive tract. The vitellaria are voluminous, occupying the extracecal areas behind the genital pore and the median field behind the ovary. Ducts from the two sides converge ventrally and unite behind the ootype to form a vitelline receptacle from which a common duct passes dorsally and dextrad to join the oviduct as described above. There is no seminal receptacle, but the initial portion of the uterus is filled with masses of spermatozoa. The eggs are oval, operculate, and relatively few. Recently extruded eggs in Ringer's solution, without a coverglass, measured 0.10 to 0.11 mm in length and 0.069 to 0.075 mm in width. The ovum is about 0.02 mm in diameter and is located near the opercular end of the egg.

The eggs are passed in the one-celled condition and at laboratory temperatures developed active, ciliated miracidia in one month. Although no life histories have been determined in the family MICROSCAPHIDIIDAE, the appearance of young worms

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Dictyangium chelydrae n. g., n. sp.

FIG. 1. Type specimen, 3.95 mm long, whole mount, ventral view.

FIG. 2. Anterior sucker, ventral view, to show details of structure.

FIG. 3. Juvenile specimen, same magnification as Fig. 1.

FIG. 4. Juvenile specimen, with rudimentary reproductive organs, same magnification as Fig. 3.

FIG. 5. Cross section, showing details of the "rosette."

FIG. 6. Reconstruction of three sagittal sections, showing genital pore and terminal portions of the reproductive ducts.

eb	excretory bladder	sv	seminal vesicle
eg	egg in genital atrium	ts	testis
ep	excretory pore	ut	uterus
gp	genital pore	vd	vas deferens
ov	ovary	vi	vitelline duct
sg	shell gland	vt	vitellaria

(Fig. 3) recovered from the turtle, suggests that the cercariae are pigmented monostomes. In these young specimens the excretory vesicle has attained its definitive form, although it probably is not developed in the cercaria.

Dictyangium may be distinguished from *Octangium* Looss, *Octangioides* Price and *Hexangitrema* Price by the absence of a posterior median notch, from *Deuterobaris* Looss by the form of the sucker and the absence of rows of ventral glands, from *Parabaris* Travassos by the location of vitellaria and uterine coils, from *Hexangium* Goto and Ozaki by the absence of cirrus sac and location of the gonads, and from *Microscaphidium* Looss, *Angiodictyum* Looss and *Polyangium* Looss by the absence of lymph vessels and differences in the form of the excretory system.

DISCUSSION

Members of the family MICROSCAPHIDIIDAE appear to be limited to tropical or subtropical regions, and infect both marine and fresh-water turtles and fishes. Since the final hosts of *Dictyangium chelydrae* have a much wider distribution than the parasite, it seems probable that the range of the fluke is limited by that of its intermediate host or hosts. Looss found these trematodes only in adult turtles, and since in the present study only large snapping turtles were examined, no information is yet available concerning the size of the turtles when the infection is acquired. Seasonal variation in egg production and occasional degeneration of the gonads were discussed by Looss (1902) and other authors, but the final explanation of these phenomena will probably not be forthcoming until the life history is known.

The family MICROSCAPHIDIIDAE (ANGIODICTYIDAE) as constituted by Price (1937) contained nine genera, to which *Dictyangium* should be added. These trematodes, although constructed on the same morphological pattern, show marked structural variation. The posterior end of the body may be rounded or manifest a distinct notch. With the exception of *Distomum quadrangulatum* Daday, 1907, they are monostomes, and the inclusion of *D. quadrangulatum* in the genus *Parabaris* by Travassos (1934) is questionable. Daday (1907) described and figured an acetabulum, 0.13 mm in diameter, situated immediately behind the genital pore, and if the original account is correct, (its accuracy was questioned by Odhner, 1911) the specimen described by Daday is probably not congeneric with *Parabaris parabaris*. In the family, the anterior sucker may be simple, may bear ventrolateral muscular lappets which recall those on the acetabulum of *Zygocotyle*, or may possess retrodorsal evaginations similar to those of many amphistomes. The esophagus may or may not have a posterior muscular thickening. The digestive ceca may extend almost to the posterior end of the body or terminate anterior to the gonads. The anterior sucker of the microscaphids is probably homologous with that of the amphistomes and of the pronocephalids, but there is much difference of opinion whether it is the equivalent of the oral sucker or the pharynx of other trematodes. Looss (1902) reviewed the subject and argued that this anterior sucker is an oral sucker and that it is not homologous with the pharynx. The muscular thickening at the posterior end of the esophagus he designated as a pharynx. This opinion was accepted by most subsequent helminthologists until Näsmark (1937) reopened the question. He stated, p. 354, "A detailed anatomical or histological comparison shows that the 'oral sucker' of the Paramphistomides is in principle constructed on the same anatomical scheme as that found in the pharynx bulbosus of the

rhabdocoelous Turbellarians, and the pharynx of the monogenous Trematodes." He regarded the "oral sucker" of other trematodes as a secondarily acquired organ, situated in front of the pharynx, and derived from the walls of the prepharyngeal part of the oral cavity. Accordingly, he stated that the amphistomes have no "oral sucker" and the anterior sucker was designated as the pharynx. Willey (1941) regarded the evidence of Näsmark as incomplete and not convincing. The problem is a difficult one, requires the consideration of extensive data, and is actually outside the scope of the present paper. Accordingly, to avoid predilection, the organ is here described as the anterior sucker. Associated with the problem of the oral sucker is that of the esophageal enlargement or bulb, situated at or near the posterior end of the esophagus. It was termed "pharynxbulbus" by Odhner (1911) who stated, "Ich verwende diese Bezeichnung, weil es mir doch nicht so ganz sicher erscheint, dass es sich hier um ein dem gewöhnlichen Distomenpharynx homologes Organ handelt. Auch wenn es so wäre, könnte ubrigens der ziemlich verschiedene Bau einen besonderen Namen rechtfertigen; der Oesophagus müsste aber dann konsequenterweise als Präpharynx bezeichnet werden." This structure, usually but not always present in amphistomes and microscaphids, consists essentially of a thickening of the muscular wall, especially the circular fibers of the region. As noted by Odhner and subsequent authors, its structure is very different from that of the pharynx of other flatworms.

Another problem is presented by the excretory and lymph vessels in members of the family. The vesicular portion of the excretory system may consist of from two to eight branching longitudinal vessels and a lymph system may or may not be present. There is still uncertainty concerning the relations of the excretory and lymphatic channels. These vessels were regarded as portions of the excretory system until Looss (1902) recognized the lymph vessels as a distinct and independent system. Earlier accounts were reviewed and new observations on lymph systems were reported by Willey (1930a, 1933). Willey (1930b) pointed out that the excretory system of *Hexangium sigani* as described by Goto and Ozaki (1929) differs from that of previously described members of the ANGIODICTYIDAE, while in structure and distribution it recalls the lymph vessel system of these trematodes. He suggested the possibility that the three pairs of longitudinal vessels in *Hexangium* may be lymph channels. Later, Willey (1935) described the excretory system of *Typhlocoelum cucumerinum*. On each side there are three main longitudinal vessels which unite posteriorly and a common stem communicates with the excretory bladder, an arrangement similar to that in *Hexangium*. The vessels give off numerous branches that form a reticulum. Histologically the vessels have membranous walls and they contain a granular coagulum in which lymphocytes are suspended. He stated, "Many features of the excretory system of *Typhlocoelum cucumerinum* are similar to those found in the lymph system of amphistomes. The intimate association with the intestine, the cuticular walls of the vessels, the granular coagulum in the lumen, and the presence of numerous cellular elements comparable to the 'lymphocytes' strongly suggest a lymph system. However, since the vessels communicate with the excretory vesicle, the lumen of which contains the same elements, and since no other excretory organs are to be found, the structure must be interpreted as an excretory system." Ozaki (1937) redescribed the excretory system of *Hexangium sigani;* in addition to the three pairs of excretory vessels, he

reported the presence of three pairs of lymph channels in this species. Although Looss (1902) described a lymph system in all of the microscaphids studied by him and stated that such a system is characteristic of the family, lymph vessels were not observed by Price in *Hexangitrema pomacanthi* and they are absent in *Dictyangium chelydrae*. A detailed study of the development of the lymph system in those species in which it occurs should provide the basis for a correct interpretation of the relation between excretory and lymphatic vessels.

SUMMARY

The family name ANGIODICTYIDAE Looss, 1902 is invalid and should be suppressed in favor of MICROSCAPHIDIIDAE Travassos, 1922. *Dictyangium chelydrae* n. g., n. sp., a member of the family, is described from *Chelydra serpentina* in Louisiana. Morphological similarities between the microscaphids and amphistomes are discussed. The absence of a lymph system in *D. chelydrae* necessitates a change in the diagnosis of the MICROSCAPHIDIIDAE. Relations between the lymph and excretory systems are considered.

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PRELIMINARY OBSERVATIONS ON THE EFFICACY OF A PRODUCT FROM OIL OF ROSE GERANIUM FOR THE REMOVAL OF INTESTINAL PARASITES FROM DOGS

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Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture

In preliminary tests of several substances for anthelmintic action, one, a product from oil of rose geranium, gave promising results in dogs, both with respect to its anthelmintic action and its apparent lack of toxicity.

The product was prepared from a sample of oil of rose geranium of domestic origin furnished by the Bureau of Plant Industry. Geraniol was removed by means of calcium chloride, and the remaining part was treated with phthalic anhydride. The acid phthalate thus formed was extracted with sodium carbonate. The sodium acid phthalate was saponified with potassium hydroxide and the liberated alcohol separated by steam distillation. The product was then distilled under reduced pressure. It had a boiling point of 111 to 117° C, p = 12 mm; density at 25° C (g per cc), 0.8609; refractive index $n\frac{25°}{D}$, 1.4557; optical rotation (Alpha) $\frac{20°}{D}$, 0°.

When analyzed by the formylation method, the substance had a citronellol content of about 92 per cent. The remaining 8 per cent presumably consisted of the alcohols found in oil of rose geranium, other than geraniol.

So far as could be determined, the literature contains no reference to the anthelmintic action of the product used in these studies, although Rico (1929, Compt. Rend. Soc. Biol., Paris, **102**: 218) reported that a substance regarded by him as geraniol gave encouraging results against hookworms when administered to two dogs.

The present tests on the anthelmintic action of this compound were conducted at the station of the Zoological Division, Beltsville Research Center, Beltsville, Maryland. The drug was administered in gelatin capsules to dogs after a fasting period of 18 hours. When a purgative was used it was given from 1½ to 2 hours after treatment, and consisted of one ounce of castor oil. The feces were examined daily for parasites in the usual manner. When no additional worms were eliminated, the animals were destroyed and the intestinal tracts examined for the presence of parasites. The usual length of time from the treatment until necropsy was 4 days.

The results obtained are shown in Table 1. Two species of ascarids were present in these dogs, *Toxascaris leonina* and *Toxocara canis,* both of which were removed by comparatively small doses of the drug. Of the two species of hookworms that were present, *Ancylostoma caninum* and *Uncinaria stenocephala,* the latter was removed by small amounts of the drug while *Ancylostoma caninum* appeared to be more resistant and required larger doses. In order to effect the removal of whip-

Received for publication, August 19, 1942.

worms, *Trichuris vulpis*, it was necessary to increase the dose rate even more. The use of a purgative did not appear to increase the efficacy or safety of the drug.

From the limited information obtained, dosages of from 0.5 cc to 0.7 cc per pound of body weight seemed to be adequate for the removal of ascarids and hookworms from dogs, although the data are insufficient to recommend this dose rate for other than experimental purposes. At no time during the course of these experiments were any clinical symptoms of toxicity evidenced by the dogs treated, nor were any lesions observed at autopsy that could be attributed to the drug. Moreover, unlike the majority of anthelmintic drugs, there is not the factor of an un-

TABLE 1.—*Data on anthelmintic tests in dogs with a product obtained from oil of rose geranium*

Dog No.	Weight (pounds)	Amount of drug given (also purgative if used)	Parasites passed	Parasites found post mortem	Efficacy (per cent)
60	22	5 cc	3 ascarids 13 hookworms	0 ascarids 2 hookworms	100 87
62	19	5 cc plus one ounce of castor oil	13 ascarids 29 hookworms 21 whipworms	0 ascarids 9 hookworms 200 whipworms	100 76 10
51	12	5 cc	4 ascarids 3 hookworms	0 ascarids 0 hookworms	100 100
52	15	5 cc	2 ascarids 1 *Taenia hydatigena*	0 ascarids 0 tapeworm	100 100
61	12	1 cc	2 ascarids 1 hookworm	0 ascarids 0 hookworms	100 100
53	10	2.5 cc	5 ascarids 2 hookworms 1 whipworm	0 ascarids 0 hookworms 3 whipworms	100 100 25
58	18	17.5 cc plus one ounce of castor oil	1 ascarid 233 hookworms 247 whipworms	0 ascarids 0 hookworms 116 whipworms	100 100 68
43	26	20 cc plus one ounce of castor oil	2 hookworms 12 whipworms	0 hookworms 29 whipworms	100 30
48	27	25 cc plus one ounce of castor oil	1 ascarid 3 hookworms	0 ascarids 0 hookworms	100 100
66	12	15 cc	1 hookworm 3 whipworms	0 hookworms 24 whipworms 8 *Dipylidium caninum*	100 20 0
69	25	12.5 cc plus one ounce of castor oil	11 ascarids 89 hookworms	0 ascarids 0 hookworms 30 whipworms	100 100 0
70	20	10 cc	4 ascarids 10 hookworms 2 whipworms	0 ascarids 0 hookworms 20 whipworms 46 *Dipylidium caninum*	100 100 10 0
46	40	5 cc plus one ounce of castor oil	3 hookworms	0 hookworms	100
67*	30	7.5 cc	none	none	?
68*	25	12.5 cc	none	none	?

* Although these dogs passed no worms after treatment and none were found on postmortem examination, hookworm ova were present in the feces before treatment and were absent four days afterwards.

pleasant odor; rather this preparation possesses a fragrance of rose perfume. It is possible that this drug may compare favorably with other anthelmintics for dogs, and may prove useful as a possible substitute for the increasingly scarce chlorinated hydrocarbons that are now widely used.

This product is not widely available at present, but could probably be made so if further experience justified its use. At a dose rate of 0.5 cc per pound of body weight it failed to remove ascarids satisfactorily from one pig; other than this single trial no data have been obtained on its possible usefulness for the removal of parasites from other host animals.

Summarizing the data herein, it will be noted that in doses varying from 1 to 25 cc per dog, a substance consisting largely of citronellol removed 100 per cent of 46 ascarids from 10 dogs, 97 per cent of 400 hookworms from 12 dogs, and 40 per cent of 708 whipworms from 7 dogs.

RESEARCH NOTES

THE RELATIVE INCIDENCE OF BLOOD PARASITES IN SOME BIRDS FROM GEORGIA

The data reported here are the results of a survey dealing with the relative incidence of blood parasites in various species of wild birds trapped in Georgia during the last four years.

The birds were trapped throughout the year, with all but 8 being taken in Bulloch County. Diagnosis for the presence of parasites was based upon a 10-minute examination of a single stained blood smear.

Results: In 275 birds belonging to 14 families and 23 species 20% of the total number of birds examined were infected with one or more genera of parasites. Mixed infections were observed in 16 cases, giving a total of 84 infections in the 55 infected birds. The percentage of birds infected with each type of parasite was as follows: *Plasmodium*, 4%; *Haemoproteus*, 9.5%; *Leucocytozoon*, 6%; *Trypanosoma*, 4%; and microfilariae, 3%. The species of infected birds, the parasites they harbored, and the number of birds in each species microscopically negative are given in Table 1.

Experimental: It has been shown by Manwell and Herman (1935, Am. J. Trop. Med. **15**: 661) that infections of *Plasmodium* are more readily detected by inoculating blood into clean canaries than by simply examining blood smears. In the present study 39 inoculations have been made into microscopically negative birds of the same species as the birds being examined. This method revealed an incidence of 31% among birds not showing patent plasmodial infections. The infections encountered by using this technique, along with symbols to indicate the new host records for the various parasites are also given in Table 1.

Multiple infections were encountered in 17 birds; these are summarized in the following tabulation in which the abbreviations used are *Haemoproteus* (H), *Leucocytozoon* (L), *Plasmodium* (P), *Trypanosoma* (T), microfilariae (M). H, L—1 dove, 1 blue jay, 1 vesper sparrow; H, M—2 blue jays; H, T—1 cardinal; H, T, M—1 blue jay; H, L, M—2 blue jays; H, P, L—1 blue jay; H, L, T, M—1 blue jay; P, L—1 cowbird; P, T—1 cowbird, 1 brown thrasher; P, L, M—1 blue jay; P, T, M—1 blue jay; P (2 species)—1 cardinal.

No blood parasites were found in 11 species of birds in which from 1 to 3 specimens were examined.

Comparison with results of other surveys: Several surveys of avian blood parasites have been made in widely separated localities, and as the number of these is extended it should be possible to describe the geographic distribution of the various species of blood parasites. The species of birds and the number of specimens in each species have varied greatly. Since it is readily apparent from these studies that the various species of birds show much variation in both the kind and number of blood parasites, the results may best be compared on a basis of the species of hosts examined rather than upon the total number of parasites encountered in all birds examined. The comparisons that can be made are not entirely satisfactory inasmuch as the results are subject to the distorting effects of incomplete sampling and possibly of variations in the seasons during which the smears were collected. Comparisons are made with the following studies: Huff (1939, J. Am. Vet. Med. Assn. **44**: 615) mainly in the Middle West, Manwell and Herman (1935, Am. J. Trop. Med. **15**: 661) in upper New York State, Herman (1938, Tr. Am. Micr. Soc. **57**: 132) on Cape Cod, and Wetmore (1940, J. Parasitol. **27**: 379) in the District of Columbia.

English sparrows: Huff (in 125 specimens) found *Plasmodium* in 10%, with 4% of the total number harboring *P. cathemerium;* Manwell and Herman (in 245 birds) found *Plasmodium* in 3%, all of which were *P. relictum.* Thus *P. cathemerium* was the predominant species of *Plasmodium* in the birds examined by Huff but was not encountered in the studies made in New York and Georgia.

White-throated sparrows: Huff observed *Haemoproteus* in 40% of the 21 birds examined; Wetmore found *Haemoproteus* in 31% and *Leucocytozoon* in 15% of 19 birds examined. Neither of these parasites was seen in 16 specimens taken in Georgia.

Starlings: In the various surveys only *Plasmodium* has been recorded and these infections have been encountered rarely. Manwell and Herman found one infection in 120 birds; Wetmore found two infections in 32 birds; and Huff examined 11 birds with negative results.

Blue jays: This bird is evidently one of the most commonly infected. Huff observed *Haemoproteus* in 44% (43 examined); Wetmore found *Haemoproteus* in 17%, *Leucocytozoon* in 14%, *Plasmodium* in 3% (29 examined). In the Georgia study 27 birds (trapped mainly in the spring) harbored a total of 40 infections with *Haemoproteus* occurring in 52%; the infections also included trypanosomes and microfilariae which were not reported in the studies by Huff and by Wetmore.

TABLE 1.—*Species of birds infected with one or more blood parasites; also number of individuals examined and number of individuals uninfected of these species*

Hosts		Parasites												
		Plasmodium												
Family and scientific name	Common name	*circumflexum*	*elongatum*	*hexamerium*	*relictum*	*vaughani*	Undetermined	*Haemoproteus*	*Leucocytozoon*	Haemosporidia (Genus undetermined)	*Trypanosoma*	Microfilariae	Number examined	Number negative
COLUMBIDAE														
Zenaidura macroura carolinensis	Mourning dove	..	..	..	..	..	..	2	2	..	..	..	6	3
COMPSOTHLYPIDAE														
Dendroica caerulescens	Black-throated blue warbler	..	..	..	..	..	..	..	..	..	..	1	1	0
CORVIDAE														
Cyanocitta c. cristata	Blue jay	2*	..	1*	..	..	1†	14	7	1	6	8	27	7
FRINGILLIDAE														
Richmondena c. cardinalis	Cardinal	1*†	1*†	..	1*†	..	..	2	1	..	2	..	7	4
Pipilo erythrophthalmus	Red-eyed towhee	..	..	..	..	..	..	..	1	..	..	..	5	4
Zonotrichia albicollis	White-throated sparrow	1*†	..	..	..	..	1†	..	..	..	..	..	16	14
ICTERIDAE														
Molothrus a. ater	Cowbird	..	..	..	2†	..	2†	..	3	1	2	..	66	60
Poecetes gramineus	Vesper sparrow	..	..	..	1*	..	..	6	2*	1	2	..	30	19
MIMIDAE														
Mimus polyglottus	Mockingbird	..	..	..	..	..	..	2*	..	1	..	..	13	10
Toxostoma rufum	Brown thrasher	2*	..	..	..	..	1	..	..	..	1	..	9	6
PLOCEIDAE														
Passer d. domesticus	English sparrow	..	..	..	2	..	1	..	..	..	..	..	41	38
STURNIDAE														
Sturnus vulgaris	Starling	..	..	..	..	1	..	..	..	..	..	..	40	39
Totals (exclusive of experimental results)		11						26	16	4	13	9	245	190

* New host record.
† Discovered by subinoculation.

Vesper sparrows: Herman examined 31 specimens during the summer months and found only a single infection of *Haemoproteus* and of *Plasmodium*; while approximately the same number of birds from Georgia examined during the spring showed a greater total number of infections, including *Leucocytozoon* and *Trypanosoma*.

Brown thrashers: Huff found *Haemoproteus* in 29 of 48 birds examined; Wetmore observed this genus in 5 of 12 birds examined; while 9 specimens in the present study were negative.

Cowbirds: Herman examined 378 birds during the summer months with the following results: *Plasmodium* in 14, Leucocytozoon in 2, Plasmodidae in 3. These results differ from those of the present (in which all the cowbirds were examined during the winter) in a higher incidence of *Plasmodium*, a lower incidence of *Leucocytozoon*, and the absence of *Trypanosoma*.—PAUL E. THOMPSON, *Georgia Teachers College*.

OPECOELINA PHARYNMAGNA N. SP. (TREMATODA) FROM THE CHINA ROCKFISH

On August 8, 1938, a single specimen of a trematode of the genus *Opecoelina*, family Allocreadiidae, was found in the dissecting dish containing the stomach and intestine of a China rockfish, *Sebastodes nebulosus*. The host was taken from the Pacific Ocean, near Fort Ross, California.

The genus *Opecoelina* was erected by Manter (1934, Carnegie Inst. Wash. Pub. 435, Papers Tortugas Lab. 28: 257–345), based upon the descriptions of two new species, *Opecoelina scorpaenae* and *O. helicoleni*. A third species, *O. theragrae*, was added to the genus by Lloyd (1938, J. Parasitol. **24**: 103–133). The specimen from the China rockfish appears to represent an undescribed species. For this trematode, the name *Opecoelina pharynmagna*, is proposed.

Opecoelina pharynmagna n. sp.
(Fig. 1.)

Body smooth, elongate, tapering at both extremities; length 3.6 mm. Body length can be separated into four approximately equal portions or units. First unit extends from oral sucker to posterior border of acetabulum; second unit extends to anterior margin of ovary and contains uterus; third unit extends to region just posterior to testes and contains the gonads; fourth unit extending to posterior end of body, occupied largely by posterior vitellaria.

FIG. 1. *Opecoelina pharynmagna* n. sp., ventral view.

Greatest width, 0.53 mm through acetabular region. Behind acetabulum body constricts abruptly; at this point body width measures 0.4 mm; from this point lateral margins remain parallel to region of ovary and then gradually taper to posterior extremity.

Oral sucker terminal, mouth ventral, length 0.179 mm, width 0.162 mm; acetabulum wider than long, width 0.36 mm, length 0.3 mm. Prepharynx very short; pharynx nearly as large as oral sucker (0.136 mm × 0.125 mm); esophagus shorter than pharynx. Intestinal biturcation approximately 0.14 mm from anterior border of acetabulum; ceca unite in posterior end of body to form short rectum. Anal opening could not be ascertained with certainty; due to expanded character of ceca in this region, it was not, at first, recognized that union existed.

Testes elongate, roughly oval, tandem, approximately 0.27 mm long and 0.185 mm wide. Ovary, four lobed, its posterior margin approaching anterior testis, its anterior margin marking equator of two body halves. Seminal receptacle and yolk reservoir dorsal, and to left of ovary; small portion of seminal receptacle overlapping margin of ovary. Laurer's canal present and to left of mid-line. Vitellarian follicles large and round, extending from point just back of acetabulum, to posterior tip of body, filling all available space, with exception of small area at ovarian level. Cirrus pouch long, sinuous, passing to the left of the acetabulum and ending in the midline, 0.256 mm

behind acetabulum. Seminal vesicle enclosed by cirrus sac. Genital pore to left and slightly anterior to bifurcation of ceca. Uterus confined to intracecal area between ovary and acetabulum. Eggs measure 77–92 microns × 43–52 microns.

Host: Sebastodes nebulosus (Ayres).

Habitat: Stomach or intestine.

Locality: Fort Ross, California.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36866.

Opecoelina pharyngmagna presents many characteristics similar to the other members of the genus. In body size and form it is very similar to *O. heliconi* Manter and *O. rhenopae* Lloyd. It differs from all the other species in having a very large pharynx, which is nearly equal to the size of the oral sucker, and a much shorter oesophagus. Like *O. rhenopae*, the seminal vesicle is enclosed in the cirrus pouch, whereas an external seminal vesicle is present in *O. heliconi* and *O. scorpaenae*.—R. F. Annereaux, *Animal Pathology Laboratory, California State Department of Agriculture.*

A REDESCRIPTION OF *CONTRACAECUM HABENA* (LINTON, 1900) LINTON, 1934

Numerous ascarids collected from *Opsanus tau* at the mouth of the Patuxent River in June, 1941, and sent to the writer for identification, proved upon comparison with the type specimens of *Ascaris habena* Linton, 1900, to belong to that species. Linton originally described this worm from *Opsanus tau* at Wood's Hole, where he reported it to be of frequent occurrence. In 1934 (James Johnstone Mem. Vol., Liverpool) he referred it to its correct genus, *Contracaecum*. Since Linton's description is inadequate, the following redescription is given:

Contracaecum habena (Linton, 1900) Linton, 1934

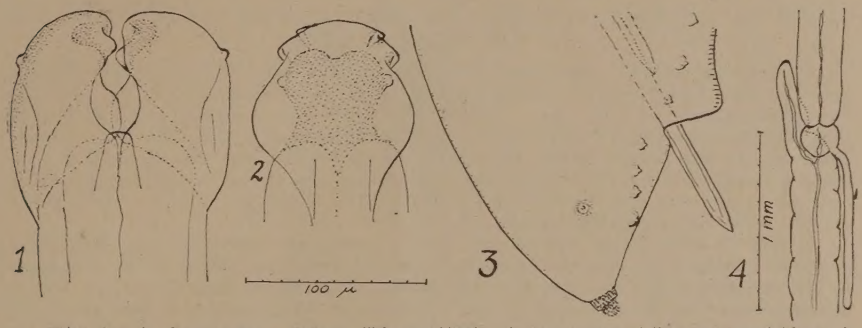
Body tapering anteriorly, reaching greatest diameter near posterior end. Cuticle finely and inconspicuously striated. Greatest diameter through lips 100 to 160 μ, about equal to length of latero-ventral lips; latero-ventral lips each have three interlocking rounded lobes situated one behind the other, the middle one, antero-posteriorly, being most prominent. Dorsal lip slightly shorter than latero-ventral lips, about 90 to 150 μ long; greatest width, slightly posterior to middle, about 10% less than length; an inner flap, forming anterior margin, rounded in front and with two pairs of lateral angles, one pair distal to the other. Pulp of dorsal lip forms two rounded lobes in front, with the papillae situated on their lateral curvatures. Interlabia 30 to 40 μ long, 30 μ wide at base, 15 μ at tip. Esophagus 4.6 to 7.8 mm long, 20 to 25% of length of body. Nerve ring 450 to 600 μ from anterior end. Esophageal ventriculus slightly broader than long, and a little broader than terminal portion of esophagus, measuring about 150 to 180 μ in length and 200 to 250 μ in width. Esophageal appendage about 740 to 970 μ long; intestinal cecum always shorter and less slender, 320 to 580 μ long. Tail in both sexes covered at tip by minute spines.

Male 16 to 30 mm long; maximum diameter 420 to 650 μ. About 24 pairs of small preanal papillae extending for distance of 0.8 to 1.2 mm anterior to cloaca; posterior 16 to 18 of them more ventral in position than the more anterior ones. Papillae near cloaca spaced about 20 to 30 μ apart, the more anterior ones about 90 to 100 μ apart. 4 pairs of ventral postanal papillae; last one largest, situated slightly beyond middle of length of tail and more ventral than others. 1 pair of laterally placed postanal papillae. Body diameter abruptly narrowed behind cloaca; tail 110 to 120 μ long, its tip provided with a spiny, retractile, button-like structure. Spicules 2.7 to 4.2 mm long, about 11% to 15% of body length.

Female 23 to 42 mm long. Maximum diameter 0.58 to 1 mm (1.5 according to Linton). Vulva 38% to 40% of body length from anterior end. Tail 240 to 250 μ long, with retractile, spiny tip.

The species of *Contracaecum* occurring as adults in fishes, unlike those in birds, are strikingly variable with respect to the absolute and relative lengths of the intestinal cecum and the esophageal appendix; whereas in the bird species the intestinal cecum is always long and invariably exceeds the esophageal appendix in length, in the fish species the reverse is true in many instances, and the intestinal cecum may be greatly reduced. The present species belongs with a group (*auctum*, *cornutum*, *fabri*, *habena*, *legendrei*, and *trichiuri*) in which the cecum is only 0.2 to 0.6 mm long, and is exceeded in length by the appendix in all except *auctum*, in which the two diverticula have about the same length. In *cornutum*, *fabri* and *legendrei* the cecum is reduced to a pocket little longer than it is wide, whereas in *auctum*, *habena* and *trichiuri* it is between 0.3 and 0.6 mm long

These three species are also characterized by having the esophageal appendix less than 1 mm in length. *C. habena* has a relatively longer esophagus and longer spicules than any other species of *Contracaecum* from fishes. Within the group characterized by a short intestinal cecum it most nearly resembles *auctum* in the shape of the lips, and resembles that species in having a spiny tail. Stefanski (1936) reported a *Contracaecum* from *Trachinus draco* which he doubtfully referred to *auctum*, pointing out that it differed from the description of that species as given by Schneider



FIGS. 1 to 3: *Contracaecum habena* (Linton, 1900). 1, latero-ventral lips and interlabium; 2, dorsal lip; 3, posterior end of male. Fig. 4. Region of junction of esophagus and intestine, showing ventriculus, esophageal appendix and intestinal cecum.

(1866) in having prominent cervical alae (a feature shared by *C. trichiuri*) and symmetrical latero-ventral lips. *C. habena* does not correspond with Stefanski's *"auctum"* in these respects, but it can be differentiated from Schneider's meager description of *auctum* only by its narrower dorsal lip with its differently shaped pulp.

Of the three specimens of *Ascaris habena*, two females and one male (U. S. Nat. Mus. Helm. Coll. No. 6533), which Linton designated types of this species, the male is hereby designated the lectotype.—ASA C. CHANDLER, *The Rice Institute, Houston, Texas.*

A CASE OF CANINE STRONGYLOIDIASIS IN TEXAS

According to a review of the literature by Augustine (1940) cases of natural canine strongyloidiasis are rare, having been reported by Fülleborn (Arch. f. Schiffs-u. Tropen-Hyg. **5**: 26–80) from China; Ware (1923, J. Comp. Path. and Therap. **36**: 108) from India; Faust (1933, Am. J. Hyg. **18**: 114–132; 1935, Arch. Path. **19**: 769–806) from New Orleans; Whitney (1936, Vet. Med. **31**: 104–105) from eastern United States (the correctness of which is questioned); Augustine and Davey (1939, J. Parasitol, **25**: 117–119) from Boston; and Lucker (1942, Vet. Med. **37**: 128–137) from Atlantic coast states. On the basis of geographic distribution and epidemiology, and of very limited cross-infection experiments, Augustine concluded that the dog strain is distinct from the human and should be recognized as *Strongyloides canis* Brumpt, 1922.

On Feb. 2, 1942, a stool from a 5-months-old cocker spaniel, consisting of a large amount of mucus and some blood, was found to contain numerous eggs of *Strongyloides* in various stages of development, together with a few hatched larvae. The dog was reported to have had dysenteric stools for several weeks, and continued to have them. In fresh stools only unhatched eggs were found. In cultures only direct development was seen. Five thousand filariform larvae were inoculated by subcutaneous injection into each of two dogs, 2500 into a rabbit, 2000 apiece into a rat, a guinea pig, and two young cats, and 800 into a mouse. None of the rodents ever showed any infection; this conforms with results obtained by Augustine and Davey (l.c.) and indicates that the dog parasite is not a rodent strain in an abnormal host. Cultures from both of the dogs were positive on the 11th day; one of the dogs escaped a few days later, but the other remained infected for about 10 weeks and then became negative, in spite of several further injections of filariform larvae derived from this dog's cultures. One of the cats produced positive cultures on the 15th and 18th days after infection, but became negative on the 21st day and thereafter.

The naturally infected dog was treated with 1 cc of C_2Cl_4 on Feb. 10, whereupon the number of eggs and larvae became greatly reduced, and the stools were much better, but on Feb. 17 a large amount of mucus and blood was passed again. Early in March the dog was given a course of treatment with ½ gr, 1-hour Seal-Ins tablets of gentian violet at the rate of two a day for seven days, which was well tolerated. For about three weeks after treatment the dog had fairly normal

stools, but then passed another dysenteric one; fecal examination showed a small number of *Strongyloides* eggs. Another 8-day course of gentian violet was given. Again the dog's stools improved, and a week after conclusion of treatment no *Strongyloides* eggs could be found, nor had they reappeared two months later. It seems probable that the treatment given was helpful in terminating the infection, but spontaneous recovery of dogs has been reported, so this conclusion must be accepted with some reservation.—Asa C. Chandler, *Rice Institute, Houston, Texas.*

NOTES ON THE ADULTS OF *PROTOSTRONGYLUS COBURNI* IN THE LUNGS OF WHITE-TAILED DEER

In a previous study of tissue changes in the lungs of white-tailed deer accompanying natural infections with *Protostrongylus coburni,* no adult worms were encountered in the gross and microscopic examination of the lungs of 20 deer, 14 of which showed infection by the presence of eggs and first stage larvae (Goble, 1941, J. Wildlife Management **5**: 141–158). No statement concerning the host reaction to the adults could be made but the belief was expressed that the major pathology in certain *Protostrongylus* infections was due to the presence and activity of eggs and larvae rather than to the presence of adults. This opinion has since been supported by examination of the material described below.

During the year ending November 1, 1939, a number of white-tailed deer (*Odocoileus virginianus*) came to autopsy at the Laboratory of Wildlife Pathology, Delmar, N. Y. These may be advantageously treated in two groups. One group consisted of 150 essentially normal, healthy deer which died of various traumatic causes. The other group consisted of 26 deer found dead or dying in a winter deer yard which has been under study for some years in an attempt to arrive at understanding of winter losses in the Adirondack mountains. The autopsies were performed by E. L. Cheatum, Pittman-Robertson pathologist, whose report on the incidence of lungworms in New York deer is in preparation. Following gross examination a slice of tissue 30 mm thick was taken from the diaphragmatic lobe of each lung and fixed in 10% formalin. Routine microtechnique furnished 10 micron sections stained in hemalum and eosin.

Microscopic examination of sections from the lungs of the 150 deer of the first group (normal deer, killed by trauma) revealed infection with *Protostrongylus* in 30%, as evidenced by the presence of eggs and larvae, but adult worms appeared in only 1.3% (2 deer). Similar examination of the material from 26 deer of the second group (found dead or dying) revealed eggs and larvae in 100% and adults in 27% (7 deer). This group contained 18 fawns, in 7 of which adult lungworms appeared and 8 adults in which no lungworms were found. Cheatum's records on over 700 deer autopsies indicate that the incidence of *P. coburni,* obtained by examination for eggs and larvae, is over twice as great in the Adirondacks as it is in the rest of the state. In view of the difficulty encountered in finding adults, the difference in the findings in the above two groups of deer would seem to indicate that in addition to a higher incidence of lungworm infection among the winter-killed deer there was a larger worm load per animal.

It was consistently apparent from the sections showing adult worms in situ that the tissue changes present were not attributable to the presence of the adult lungworms. This is in sharp contrast to the intensive reaction sometimes induced by the arrival of fourth stage larvae in the lung from the blood stream and to the extensive lymphocytic infiltration and septal hyperplasia provoked by the presence of segmenting ova and newly hatched larvae. The fact that the pathological picture in the lungs of two deer, which were shot and immediately autopsied, was the same as in those which died or were killed and sent to the laboratory for autopsy, is regarded as evidence that there was no post-mortem migration of the adult lungworms.—Frans C. Goble, *Wildlife Research Center, N. Y. State Conservation Dept., Delmar, New York.*

NEW HOST RECORDS OF NEMATODES FROM MUSTELIDAE (CARNIVORA)

During the past two years the writer had the opportunity to autopsy 16 weasels [7 *Mustela c. cicognani* (short-tailed weasel) 5 *M. longicauda spadix* (long-tailed weasel), 2 *M. r. rixosa* (least weasel) 2 *M. frenata noveboracensis* (New York weasel)], 30 ranch-raised minks and 8 wild minks (*Mustela vison mink*), 8 skunks [5 *Mephitis hudsonica* (northern plains skunk) 3 *M. m. avia* (Illinois skunk)], 4 badgers (*Taxidea t. taxus*), and 3 martens [2 *Martes a. americana* (American marten) and 1 *M. p. pennanti* (fisher)] which were submitted or collected for examination. Some of the parasitic findings represent new host occurrences so that the results seem worthy of record.

Physaloptera maxillaris was found in the stomach of 3 short-tailed weasels (*Mustela c. cicognani*), 1 least weasel (*M. r. rixosa*) and 1 long-tailed weasel (*M. longicauda spadix*), all new host records for this nematode.

In a previous paper the writer (1941, Proc. Helm. Soc. Wash. **8**: 23–30) listed the following

North American hosts of *P. maxillaris:* Mustelidae: *Spilogale putorius* (spotted skunk), *S. g. gracilis* (canyon spotted skunk), *S. g. saxatilis* (Great Basin spotted skunk), *S. interrupta* (prairie spotted skunk), *Mephitis mephitis* (Canada skunk), *M. m. mesomelas* (Louisiana skunk), *M. m. nigra* (eastern skunk), *M. m. avia* (Illinois skunk), *M. elongata* (Florida skunk), *M. o. occidentalis* (California skunk), *M. hudsonica* (northern plains skunk), *M. macroura milleri* (northern hooded skunk), *Conepatus mesoleucus venaticus* (Arizona hog-nosed skunk), *Mustela vison mink* (common mink), *M. v. vulgivaga* (southern mink), *Taxidea t. taxus* (common badger), *T. t. berlandieri* (Mexican badger), and *T. t. neglecta* (western badger). Procyonidae: *Procyon l. lotor* (eastern raccoon) and *P. l. elucus* (Florida raccoon). Foreign hosts include: Mustelidae: *Mephitis chinche* (Brazilian skunk) Brazil, *M. suffocans* (bare-nosed skunk) Brazil, *Mydaus* sp. (Borneo badger) Borneo; Procyonidae: *Procyon cancrivora* (crab-eating raccoon) Europe, Trinidad, *Nasua narica* (coatimundi) Brazil.

Later, the writer (1941, Ibid. **8**: 63–64) listed *Mustela c. cicognani* (short-tailed weasel) and *M. frenata* (long-tailed weasel) as host of *Physaloptera* sp. Species identification was not made due to lack of mature male or female specimens, but they were probably *P. maxillaris.* This material was kindly sent to the writer by Dr. A. B. Erickson, University of Minnesota.

Molineus patens (Dujardin, 1845) was found in the small intestine of *Mustela r. rixosa* (least weasel) and *M. v. mink* (common mink). The least weasel appears to be a new host for this trichostrongylid nematode. North American records of this parasite are infrequent. Skinker (1932, J. Parasitol. **19**: 94) reported *M. patens* for the first North American records in a "mink" from Mississippi and *Canis lestis* (Great Basin coyote) from Washington. European hosts of *M. patens* include *Putorius putorius* (iltis). Germany, *Mustela nivalis* (weasel) England, *M. erminea* (ermine or stoat) England, *M. sibiricus* (Siberian weasel) Russia, *Vormela sarmatica* (marbled polecat) and *Martes foina* (beech marten), all members of the Mustelidae.

Some nematodes forwarded to the Zoological Division, Washington, D. C., from the stomachs of 2 martens, *Martes a. americana* (American marten) and *M. p. pennanti* (fisher) were identified by Dr. E. W. Price as *Soboliphyme baturini* Petrow, 1930. Price (1930, J. Parasitol. **17**: 57) reported the first North American record from Montana of this parasite in the stomach of *Gulo* sp. (wolverine), Mustelidae. The above hosts are recorded as new. Hosts reported by Petrow (1930, Zool. Anz. **86**: 265–272) from Russia were *Martes zibellina* (marten), *Vulpes vulpes* (fox), and *Felis domestica* (domestic cat).

Ascaris sp. probably *A. columnaris* Leidy, 1886, described from skunks and raccoons were recovered from the small intestine of 2 badgers (*Taxidea t. taxus*). One badger harbored a large number of *Physaloptera torquata* Leidy, 1886. Other North American hosts for this parasite are *Taxidea t. neglecta* (western badger) and *Procyon l. lotor* (eastern raccoon).—BANNER BILL MORGAN, *Department of Veterinary Science, University of Wisconsin.*

ANIMAL PARASITES OF THE FOX SQUIRREL, *SCIURUS NIGER RUFIVENTER,* IN SOUTHEASTERN KANSAS

Approximately thirty species of animal parasites have been reported from the fox squirrel, *Sciurus niger rufiventer,* from widely scattered areas but mostly from the central states. Apparently very few specimens of this host have been examined systematically for all kinds of animal parasites.

A considerable number of reports on parasites of the fox squirrel has been published, but in most instances only one or two species of parasites was studied. Most of this literature is listed by Harkema (1936, Ecol. Monogr. **6**: 151–232) and by Katz (1939, Ohio Wildlife Res. Sta., Release No. 131). Two relatively recent and notable exceptions are Katz's report of eleven species of parasites from sixteen fox squirrels in Ohio and Chandler's (1942, J. Parasitol. **28**: 135–140) report of six species of helminths from thirteen fox squirrels in southeast Texas.

One hundred fox squirrels, *Sciurus niger rufiventer,* from southeastern Kansas during the period of October, 1939, to July, 1940, were examined for all animal parasites. The chief results of this investigation are summarized in Table 1.

Of the sixteen parasites listed in the table the following have been previously reported from the fox squirrel:

Eimeria sp., *Taenia pisiformis, Heligmodendrium hassalli, Rictularia* sp., *Orchopeas wickhami, Neohaematopinus sciurinus, Dermacentor variabilis,* Sarcoptidae, and *Trombicula* sp.

Pathological conditions were observed only in the squirrel infected with the itch mite. Emaciation, deformed vertebrae, and loss of hair were observed in this animal. The maximum number of species of parasites found in any host was six. No parasites were found in seven squirrels.

TABLE 1.—*Parasites found in 100 squirrels* (Sciurus niger rufiventer) *examined from southeastern Kansas, 1939–40*

Name of parasite	Squirrels infected	Parasites per infected squirrel	
		Average	Maximum
Eimeria sp.	65	Many	Very many
Amoeba (8-nucleate cyst)	1	Very many	Very many
Coccidia	1	Few	Few
Catenotaenia sp.	2	1	1
Taenia pisiformis (larva)	1	4	4
Heligmodendrium hassalli	17	12	72
Trichostrongylus calcaratus	7	7	11
Rictularia sp.	1	2	2
Ascaris sp. (immature)	1	1	1
Hoplopsyllus affinis and *Orchopeas wickhami*	63	6	40
Neohaematopinus sciurinus	46	15	115
Dermacentor variabilis	7	2	5
Sarcoptidae	1	Very many	Very many
Trombicula sp.	15	3	21
Atricholaelaps glasgowi	1	3	3

It may be of interest to note that of the species of parasites listed in Table 1, only four are found in the list of eleven species reported by Katz. Furthermore, of the seven species of helminths listed in Table 1, the six reported by Katz, and the six reported by Chandler, only one species was included in more than one of these reports. *Heligmodendrium hassalli* is common to all three studies.

Dr. E. R. Becker, Dr. H. E. Ewing, Dr. E. W. Price, Mr. J. T. Lucker and Mr. Allen McIntosh kindly aided in the identification of the parasites.—EDWARD GRAHAM AND JACOB UHRICH, *Kansas State Teachers College, Pittsburg, Kansas.*

STRONGYLOIDES PLANICEPS, NEW NAME FOR *S. CATI* ROGERS

The name *Strongyloides planiceps* is advanced to replace the homonym *Strongyloides cati* Rogers (1939, J. Helm. 17: 229–238) in connection with a parasite found in the domestic cat but originally obtained from *Felis planiceps*. The first use of the name *S. cati* appears to be by Brumpt (1927, Précis de Parasitologie, Masson et Cie, Paris) in a footnote on page 662, "Au Bengale, le Chat presénte, dans 20 pour 100 des cas sur 250 examinés, un *Strongyloides* identifié au *stercoralis* par A. Chandler (1925) et considéré comme une espèce particulière, *S. cati*, par d'autres auteurs." Attempts to locate the "autres auteurs" have failed and it appears possible that the name *S. cati*, unaccompanied by an indication as it was first used, is a nomen nudum.

The help given by Dr. Benjamin Schwartz who pointed out that the name *S. cati* was a homonym and who has endeavored to trace the authors referred to by Brumpt, is gratefully acknowledged.—WILLIAM P. ROGERS, *Institute of Agricultural Parasitology, St. Albans, England.*

SPECIMENS NEEDED BY THE MEDICAL SCHOOLS

The Department of Parasitology and Tropical Medicine, Army Medical School, Washington, D. C., is serving as a Distributing Center for Parasitological Specimens under the auspices of a committee formed by the American Association of Medical Colleges and the National Research Council. The Department is functioning as a collecting and distributing agency for gross and microscopic specimens of protozoa, parasitic worms and ova, arthropods of medical importance, malaria and other blood parasites. If you or your institution possess any specimens which would be valuable to medical schools for these purposes and which you are willing to contribute, please send them to the Director, Army Medical School, Army Medical Center, Washington, D. C. Mailing containers and shipping directions will be furnished upon request. Your cooperation in these matters will be greatly appreciated.

GEORGE W. HUNTER, III,
Major, Sanitary Corps.